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THE INFLUENCE OF MOISTURE UPON THE RAPIDITY OF DECOMPOSITION OF LOWMOOR PEAT[1]

SELMAN A. WAKSMAN AND E. R. PURVIS[2]

New Jersey Agricultural Experiment Station

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The problem of the rapidity of decomposition of peat has certain important theoretical aspects and practical applications. Different types of peat, such as the lowmoor, forest, highmoor, and sedimentary, vary considerably in their chemical composition, in the nature of the microbial flora inhabiting these peats, and in the rapidity with which they can be attacked by microörganisms; it is, therefore, natural to expect that these peats will also vary considerably in the rapidity of their decomposition, as influenced by different environmental conditions and treatment, and in the extent of liberation of the plant nutrients in an available form.

One may use for an investigation of this nature a typical lowmoor peat, which contains about 3 per cent nitrogen or 20 per cent organic nitrogenous substances, about 5 to 10 per cent mineral constituents, about 50 per cent lignins and lignin-like complexes, about 5 to 8 per cent hemicelluloses, and a number of other substances in lower concentrations, such as fats, waxes, and cellulose. This type of peat usually has a pH of 5.0 to 6.5 and contains an abundant flora of microörganisms (2), so that as soon as it is drained, it begins to undergo rapid decomposition, without further treatment. Theoretically, it is important to determine what chemical constituents of the peat are attacked first, under these conditions, how rapidly the nitrogen becomes available, and how the conditions of decomposition affect the rate of decomposition. Practically, especially if such types of peat are not more than 2 to 3 meters deep when drained, it is essential to establish how long such peat can be cultivated and how rapidly it will tend to disappear completely, as a result of continuous drainage and cultivation, leaving only a clay, sand, or rock bottom.

Results obtained in the study of decomposition of lowmoor peat under controlled conditions need not necessarily apply to the decomposition of other types of peat, especially of highmoor peat, since the depth, chemical composition, physico-chemical nature, and microbiological population of the latter is

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[2] The authors are indebted to Mr. W. C. Douglas of the Punta Alegre Sugar Company and to Mr. J. J. Hester of the Fellsmere Corporation for supplying the samples of peat and for rendering valuable assistance in carrying out this investigation.

so distinctly different from that of other types of peat that the results will be markedly different.

Among the conditions which have a decided influence upon the decomposition of a lowmoor peat is the moisture content. This is especially true for warm regions, where the favorable annual temperature does not serve as a check upon the activities of microörganisms. It is well established that the anaerobic conditions of the peat bog, resulting from the saturation of the bog with water, are largely responsible for the formation and accumulation of the peat. It is also known that when a region is subject to a period of dryness, the surface layers of the peat bog will undergo rapid decomposition, as shown by the formation of the limiting horizon (Grenzhorizont) in the European highmoor peats. It still remains to be determined experimentally how the relative abundance of moisture will affect the rate of decomposition and whether, by

TABLE 1

Influence of moisture content upon the decomposition of a lowmoor peat, from Florida

MOISTURE CONTENT	WEIGHT OF ORIGINAL PEAT*	INCUBATION	CARBON DIOXIDE LIBERATION	NITROGEN LIBERATION		
				NH_3-N	NO_3-N	Total N
per cent	*gm.*	*days*	*mgm. C*	*mgm.*	*mgm.*	*mgm.*
78	100	54	81.0	....	6.56	.....
55	100	54	196.2	....	3.84	.....
40	100	54	89.0	....	1.95	.....
28	100	54	29.4	....	.....	
78	300	58	236.9	4.8	16.52	21.32
55	300	58	822.5	40.2	4.50	44.70
78	300	79	.....	13.2	17.17	30.37
55	300	79	.....	60.0	8.01	68.01

* The moisture content of the original peat was 78 per cent.

controlling the moisture content, one may be able to control also the speed of decomposition of the peat.

The experiments reported in this study were undertaken with the idea of obtaining further information on the problem of the influence of environmental conditions upon the rate of peat decomposition. The first experiment was carried out with peat (largely *Cladium*) obtained from the surface 30 cm. of the Fellsmere peat area in Florida. Fresh moist peat in 100-gm. and 300-gm. portions was adjusted to different moisture contents, by gradually being dried down at room temperature; the preparations were then placed in flasks, connected with the respiration apparatus and incubated at 28°C. Air, freed from CO_2, was passed over the surface of the peat and the CO_2 liberated was absorbed in standard $Ba(OH)_2$ solution. At the end of different periods of incubation, the ammonia and nitrate contents of the peat were determined. The results given in table 1 show that by reducing the moisture content to 50 or 60 per cent of the total peat, there is a rapid increase in the rate of decom-

position; from 2.5 to 3.5 times as much carbon was given off as CO_2 from the same amount of peat when the moisture was reduced from 78 to 55 per cent. It should be mentioned here that the particular peat material was taken from a layer above the water table; this peat was, therefore, partly drained, as shown by its comparatively low original moisture content, namely 78 per cent, instead of about 90 per cent moisture, which is ordinarily found in this type of peat in the undrained bog. Parallel with the liberation of the carbon as CO_2, nitrogen was liberated as ammonia and nitrate. It is of interest to note that the lower the moisture content the greater was the relative liberation and accumulation of ammonia over the nitrate. When the moisture content was reduced further, the rate of decomposition was diminished, so that, at 40 per cent moisture, the rate was about the same as at 78 per cent. At 28 per cent moisture, decomposition was markedly reduced, since the peat now became almost air-dry.

In order to study this problem in greater detail, quantities of peat were obtained from the same area, but at different depths. The peat was now taken from a cultivated field, 75 feet from a drainage sublateral, and midway between two field ditches, 40 feet apart and 18 inches deep. The layers from which the peat samples were removed were marked as follows: A, at a depth of 0–30 cm., B, at 30 to 60 cm., and C, at 60 to 75 cm. depth. The second sample was just below the surface of the water table, and the third sample was 30–45 cm. below the surface of the water table. The moisture content of A, or surface sample of peat, was 81.2 per cent, of B, or the second layer, 89.84 per cent, and of C, the third layer, 89.95 per cent; the last two layers were completely saturated with water. The peat was taken in such a manner so as to disturb it as little as possible. This was done by pressing down heavy, tin cans, 15 cm. long and about 4 cm. in diameter, with small holes in the bottom, at the particular depths of peat; the cans were then removed with the peat, closed with tight fitting tops and immediately sealed; the small holes at the other end were also closed, and the cans shipped to the laboratory. The peat was immediately removed from the cans and used for the following experiment.

Several 500-gm. quantities of the fresh peat were weighed out; some were left with the original moisture, while some were dried down, at about 50°C., so as to reduce the moisture of the peat, in order to obtain a varying water content. The peat samples, with the moisture thus adjusted, were all inoculated with a suspension of fresh garden soil, placed in liter flasks with side arms, and connected with the respiration apparatus in the incubator (28°C.). Carbon-dioxide-free air was passed over the peat and collected in standard barium hydroxide solution. The plan was to allow the peat to decompose under definite conditions for a period of 18 to 24 months. However, since as a result of decomposition, there is an accumulation of salts, which, under natural conditions, are removed by drainage or by growing plants, the accumulated salts and soluble organic substances were leached out at definite intervals of

time, so as to prevent any possible injury that they may have upon the continuous decomposition of the peat. At the end of the first and second 6-month periods, the peat samples were removed, the moisture content was determined, and all the peats were leached with distilled water. The leachings were analysed for ammonia, nitrate, total nitrogen, total organic matter, and bases. The peat samples were again adjusted to the original moisture content, again inoculated with a fresh soil suspension, and returned to their respective flasks.

At the end of 560 days' decomposition, the experiment was discontinued. The peat samples were removed and analyzed. A comparison of the composition of the peat at the beginning and at the end of the decomposition periods,

TABLE 2

Decomposition of peat at different moisture contents, at constant temperature, for a period of 560 days

On basis of original 100 gm. of dry peat

PEAT LAYER DEPTH	MOISTURE CONTENT	TOTAL DRY MATERIAL AT START	TOTAL DRY MATERIAL AT END OF DECOMPOSITION	LOSS OF MATERIAL, DUE TO DECOMPOSITION	ORGANIC MATTER LIBERATED AS CO_2 ($C \times 1.8$)	ORGANIC MATTER REMOVED IN TWO LEACHINGS	LOSS OF ORGANIC MATTER UNACCOUNTED FOR*
cm.	*per cent*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*
0–30	83.8	100	90.4	9.6	4.78	1.50	3.32
0–30	79.9	100	90.2	9.8	5.38	1.38	3.04
0–30	71.3	100	79.9	20.1	10.86	3.84	5.40
0–30	52.8	100	80.7	19.3	10.95	3.87	4.48
0–30	33.3	100	87.4	12.6	4.31	1.58	6.71
30–60	89.8	100	89.4	10.6	6.58	2.09	1.93
30–60	73.5	100	85.8	14.2	11.95	2.89	−0.64
30–60	69.5	100	83.6	16.4	14.45	2.95	−1.00
30–60	58.8	100	84.9	15.1	13.49	3.03	−1.52
60–75	90.9	100	91.2	8.8	6.55	1.98	0.27
60–75	81.3	100	75.4	24.6	18.29	3.12	3.19
60–75	72.8	100	81.1	18.9	17.35	2.98	−1.43
60–75	63.0	100	87.9	12.1	9.43	2.02	0.65

* This loss is partly accounted for by the inorganic constituents of the leachings (table 4) which were not included in these results; − indicates an excess.

as well as of the extent and nature of decomposition at the different moisture contents and of the rate of decomposition of peat taken from different depths, permits one to draw some very interesting conclusions. In order to facilitate comparisons, the results were all recalculated on the basis of 100 gm. of original dry peat material.

Table 2 gives the total loss in weight as a result of decomposition, the amount of organic matter decomposed and liberated as CO_2 (by multiplying the carbon of the CO_2 by 1.8), and the amount of organic matter made soluble and removed in the leachings after 6 and 12 months' decomposition. These results show that at certain moisture contents, namely at about 50 to 80 per cent, the decomposi-

tion of the peat is at a maximum. As much as 20 per cent of the total peat, and in one instance nearly 25 per cent of the peat, was decomposed in less than 19 months, under optimum moisture and temperature conditions. Most of the peat decomposed was lost into the atmosphere as carbon dioxide. Actually, on the average of the seven samples of peat, which had decomposed under optimum moisture conditions, about 14 per cent of the total peat was liberated as CO_2 into the atmosphere. By accounting for the moisture and the ash contents of the peat the percentage decomposition will be found to be still greater.

TABLE 3

Changes in nitrogen and ash content and in reaction of a lowmoor peat, as a result of continuous decomposition

On per cent basis of dry peat

DEPTH OF PEAT	MOISTURE CONTENT	NITROGEN CONTENT		ASH CONTENT		REACTION	
		At start	After 560 days decomposition*	At start	After 560 days decomposition*	At start	After 560 days decomposition*
cm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pH*	*pH*
0–30	83.8	3.03	3.30	9.15	10.56	6.0	5.3
0–30	79.9	3.03	3.43	9.15	10.05	6.0	5.0
0–30	71.3	3.03	3.35	9.15	9.40	6.0	4.6
0–30	52.8	3.03	3.53	9.15	10.00	6.0	4.6
0–30	33.3	3.03	3.06	9.15	10.00	6.0	5.3
30–60	89.8	3.24	3.41	5.10	5.16	5.5	4.7?
30–60	73.5	3.24	3.77	5.10	5.90	5.5	5.4
30–60	69.5	3.24	3.66	5.10	5.73	5.5	5.5
30–60	58.8	3.24	3.55	5.10	5.95	5.5	5.5
60–75	90.9	2.97	3.73	5.02	5.27	6.0	5.5
60–75	81.3	2.97	4.03	5.02	6.87	6.0	5.0
60–75	72.8	2.97	3.76	5.02	6.65	6.0	5.1
60–75	63.0	2.97	3.46	5.02	5.83	6.0	5.6

* Allowance must be made for the nitrogen and ash removed in the two leachings, as shown in table 4.

There is no doubt that when the peat is brought into the laboratory artificial conditions are created which are totally different from those prevailing in the field. However, the general principles will be found to be alike in both instances.

Table 3 shows the changes in the nitrogen, in the ash content, and in the reaction of the peat as a result of the continuous decomposition, under standard conditions. As a result of decomposition, there was a marked increase in the relative amount of nitrogen and ash. By taking into consideration the quantities of nitrogen and minerals removed in the two leachings (table 4), one can readily calculate that the greatest increases in the liberation and accumulation of nitrogen and ash corresponded to the most active decomposition of the peat. The pH values decreased, or the acidity increased, with an increase in the

decomposition, no doubt as a result of the formation of nitrates from the ammonia which resulted from the decomposition of the nitrogenous complexes of the peat.

On computing the ratio between the carbon liberated as CO_2 to the nitrogen liberated as ammonia and nitrate, it is found to be narrowest with the highest moisture content. The average ratio for all the peats is C/N = 20.5. If one considers the fact that the carbon-nitrogen ratio in the peat itself is about 18, and further the fact that this peat has only a low content of available carbohydrates (cellulose, hemicelluloses), as seen from the complete analysis of

TABLE 4

Influence of moisture content of peat on the rapidity of its decomposition, as measured by the forms of nitrogen liberated in an available form and composition of leachings

On the basis of 100 gm. of original dry peat

DEPTH OF PEAT	MOISTURE CONTENT	TOTAL NITROGEN LIBERATED*	COMPOSITION OF LEACHINGS AFTER 6 AND 12 MONTHS DECOMPOSITION			C/N†
			Total water-soluble matter	Total nitrogen	Ash	
cm.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
0–30	83.8	150.3	1.398	57.8	623	17.7
0–30	79.9	130.6	1.383	69.2	643	22.9
0–30	71.3	291.3	3.843	235.3	1,045	20.7
0–30	52.8	279.9	3.872	258.3	1,053	21.7
0–30	33.3	122.2	1.583	95.3	571	19.7
30–60	89.8	208.0	2.088	115.8	1,009	17.6
30–60	73.5	356.3	2.895	171.6	748	18.7
30–60	69.5	362.0	2.954	207.5	726	22.2
30–60	58.8	299.5	3.054	155.0	886	25.0
60–75	90.9	264.4	1.976	60.0	1,000	13.8
60–75	81.3	489.2	3.122	152.6	.....	20.8
60–75	72.8	410.5	2.982	149.1	.....	23.4
60–75	63.0	231.5	2.023	154.3	.....	22.6
Average.....						20.5

* As NH_3 and NO_3-N in the two leachings and at the final analysis.

† C = carbon liberated as CO_2; N = nitrogen liberated as NH_3 and NO_3.

the peat, one must be led to conclude that the major organic complexes, namely, the lignins and lignin-like complexes and the proteins underwent active decomposition. In the case of fresh plant residues, the contrary is true, because of the fact that these residues contain a variety of substances, some of which decompose readily, while others are resistant to decomposition. This type of peat, however, is made up largely of two organic chemical complexes (table 5), namely, the lignins and proteins, which probably exist in the peat in chemical combination; one would expect, therefore, that there should be a parallel liberation of the carbon and the nitrogen, when this peat undergoes decomposition.

The fact that the ratio of carbon to nitrogen in the decomposition products is somewhat wider than in the peat itself would tend to indicate that the residual material gradually becomes enriched in nitrogen; this was actually found to be the case, as shown in table 3. The greater the extent of decomposition of the peat, the higher is the nitrogen content of the undecomposed material.

In order to compare further the influence of the moisture content of peat, as well as the depth from which the peat was taken, upon the rate of its decomposition, one should compare the rate of decomposition, as expressed best by the curves giving the course of evolution of CO_2 (figs. 1, 2, 3). In figure 1, the data for the surface layer of peat are given. The results clearly point to the fact that the greatest decomposition of this type of peat takes place at a moisture content of 53 to 71 per cent; when the moisture drops much below 50

TABLE 5

Proximate composition of peat taken at three different depths

Per cent basis of dry material

	DEPTH OF PEAT, CM.		
	0–30	30–60	60–75
Chemical constituents:			
Ether-soluble	1.06	1.46	1.57
Cold and hot water-soluble	4.07	4.77	4.23
Alcohol-soluble	1.97	1.95	2.04
Hemicelluloses	3.03	2.81	4.10
Cellulose	Tr.	Tr.	Tr.
Lignin	50.60	51.39	48.42
Crude protein	18.94	20.25	18.56
Ash	9.15	5.10	5.02
Total	88.82	87.73	83.94

per cent of the total peat or is increased to 80 per cent or above, decomposition is materially arrested. In the case of the second layer (fig. 2), similar results were obtained, except that none of the samples were dried sufficiently to reduce the moisture below 59 per cent; however, at 90 per cent moisture, decomposition was markedly diminished. The results obtained on the decomposition of the deepest layer are similar to those found for the material taken from the surface layer; at 73 to 81 per cent moisture, decomposition was at an optimum, dropping rapidly, in course of time, with the higher or lower moisture contents.

In order to compare the rates of decomposition of the peat taken from the three different layers, the data for each peat were averaged and the averages plotted in figure 4. The results show that, during the first 8 months, the peat taken from the surface layer (partly drained) decomposed most rapidly; however, after 12 months of decomposition, the peat taken from the deepest layer

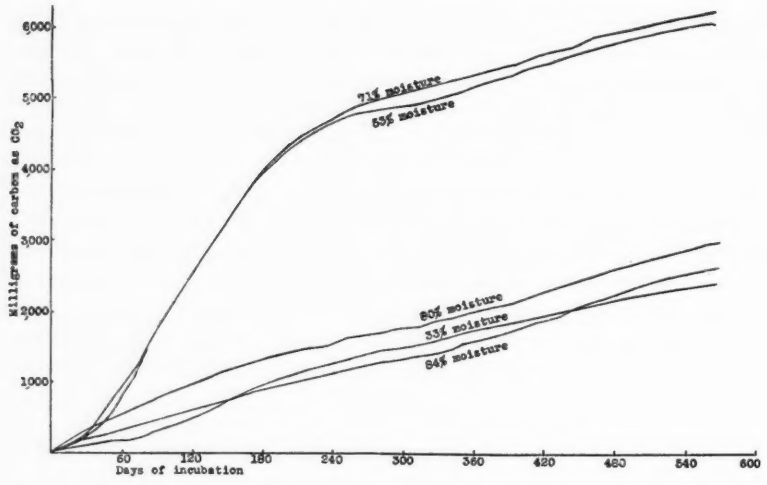

FIG. 1. INFLUENCE OF MOISTURE UPON THE EVOLUTION OF CO_2 IN THE DECOMPOSITION OF PEAT TAKEN FROM THE SURFACE 30 CM. LAYER OF A LOWMOOR FLORIDA PEAT, ON THE BASIS OF 100 GM. OF DRY PEAT

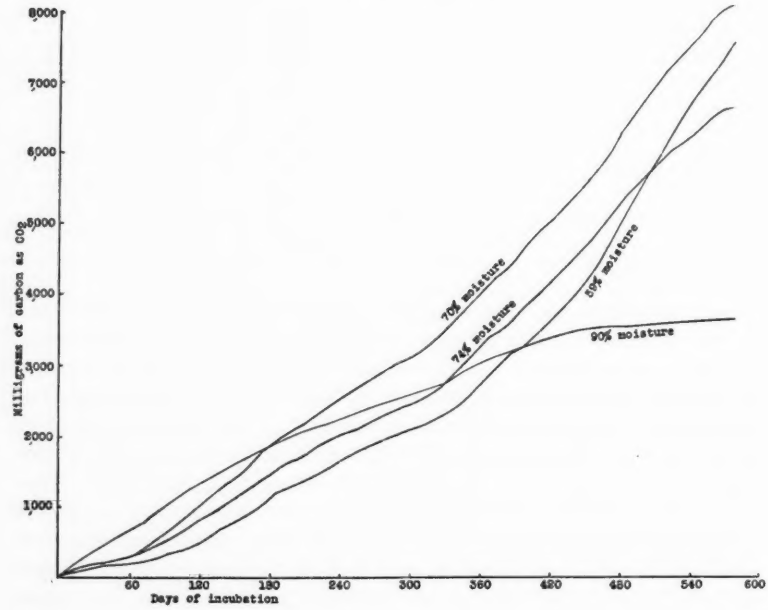

FIG. 2. INFLUENCE OF MOISTURE UPON THE EVOLUTION OF CO_2 IN THE DECOMPOSITION OF PEAT TAKEN FROM THE 30–60 CM. LAYER OF A LOWMOOR FLORIDA PEAT, ON THE BASIS OF 100 GM. OF DRY PEAT

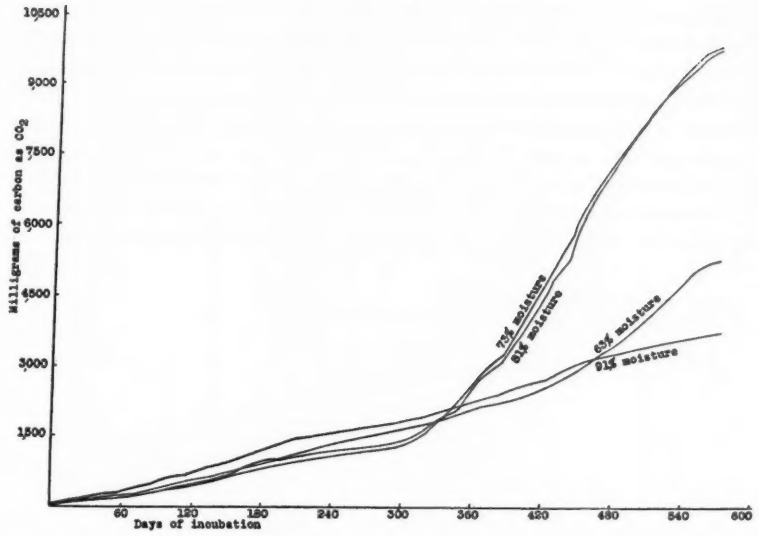

FIG. 3. INFLUENCE OF MOISTURE UPON THE EVOLUTION OF CO_2 IN THE DECOMPOSITION OF PEAT TAKEN FROM THE 60–75 CM. LAYER OF A LOWMOOR FLORIDA PEAT, ON THE BASIS OF 100 GM. OF DRY PEAT

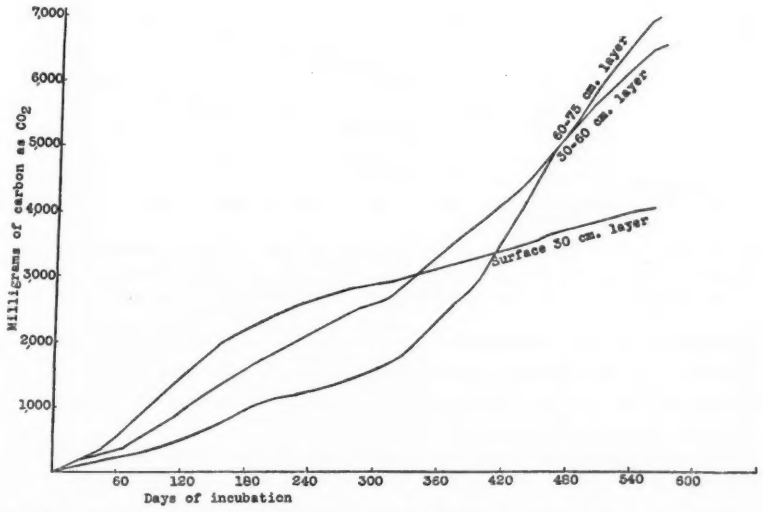

FIG. 4. COMPARATIVE RATE OF EVOLUTION OF CO_2 IN THE DECOMPOSITION OF A LOWMOOR FLORIDA PEAT, TAKEN FROM THREE DIFFERENT DEPTHS, ON THE BASIS OF 100 GM. OF DRY PEAT

underwent most active decomposition, with the middle layer coming between. The following explanation for this difference suggests itself: peat saturated for many centuries with water is in a certain reduced state, with only anaerobic bacteria finding conditions favorable for their growth and reproduction. When this peat is drained and brought in contact with atmospheric oxygen, a certain "lag period" has to pass, before the aerobic organisms are able to decompose the peat constituents actively. Whether this lag period consists in the destruction of certain substances toxic to aerobic organisms or whether it consists in the oxidation of the substances present in a reduced condition, remains to be

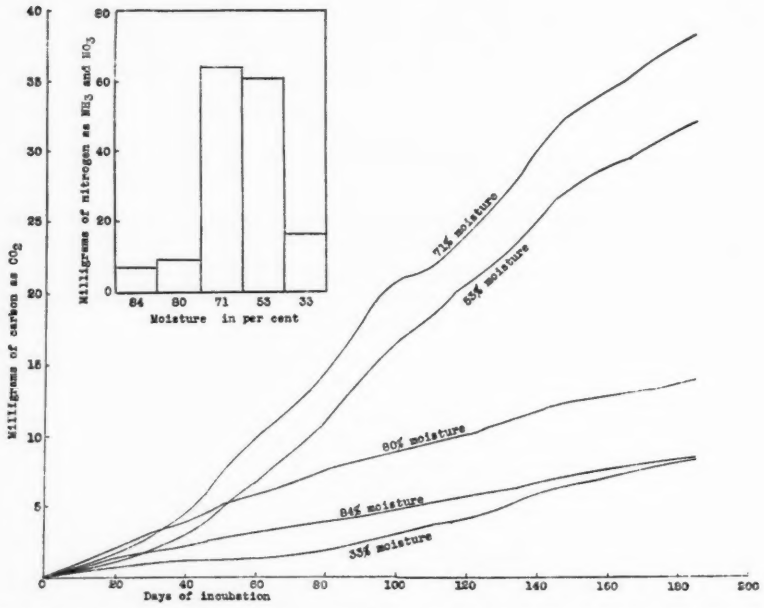

FIG. 5. INFLUENCE OF MOISTURE UPON THE DECOMPOSITION OF PEAT, TAKEN FROM THE SURFACE 30 CM. OF THE BOG, DURING THE EARLY STAGES OF DECOMPOSITION, AS SHOWN BY EVOLUTION OF CO_2 AND LIBERATION OF NITROGEN IN AN AVAILABLE FORM

determined. It may possibly be due to a gradual adaptation of the aerobic organisms to the resistant organic constituents of the peat, especially the lignins and the proteins, which were accumulated because of their resistance to attack by anaerobic bacteria.

The results presented in figure 5 illustrate the relative liberation of carbon as CO_2 and of nitrogen as ammonia and nitrate from the surface peat, during the early stages of decomposition. The two are found to run parallel, as brought out in the tables, while the influence of moisture upon the decomposition of the peat is especially emphasized.

TABLE 6

Proximate chemical composition of peat after 560 days' decomposition under different moisture conditions

Per cent of dry material

Depth of layer, cm	0–30	0–30	0–30	0–30	0–30	30–60	30–60	30–60	30–60	60–75	60–75	60–75	60–75
Moisture content	83.8	79.9	71.3	52.8	33.3	89.8	73.5	69.5	58.8	90.9	81.3	72.8	63.01
Ether-soluble	1.23	1.32	1.48	0.91	0.72	1.69	1.53	1.55	1.11	1.48	1.40	1.58	0.83
Alcohol-soluble	1.27	1.13	1.75	1.96	1.91	1.43	1.49	1.48	1.91	1.70	1.51	1.43	1.81
Cold water-soluble organic matter	0.92	0.85	1.50	1.28	0.97	2.27	2.59	3.10	3.34	0.99	7.08	5.04	2.31
Hot water-soluble organic matter	1.32	1.28	2.15	2.30	1.35	1.58	2.72	2.45	3.87	1.48	2.98	2.99	1.58
Hemicelluloses and cellulose	3.84	3.94	3.81	3.83	4.22	4.27	4.08	4.13	3.88	4.09	3.34	3.34	3.56
Lignins	55.61	58.18	56.31	54.37	55.18	53.93	50.38	50.56	48.93	58.41	46.09	48.02	52.03
Crude protein	20.09	19.93	17.90	17.65	18.31	19.95	20.08	19.82	19.84	18.25	18.04	18.91	19.38
Ash	9.07	8.88	8.48	8.93	8.88	5.40	6.31	6.87	6.22	5.78	6.99	8.11	5.68
Total	94.66	96.58	95.23	93.16	93.40	92.96	91.79	92.37	91.36	94.02	89.55	92.08	90.14

At the end of the decomposition period, the residual peat was dried and subjected to a proximate analysis. The results given in table 6 show that the various chemical constituents of the peat did not decompose with the same rate. The ether-soluble substances, which make up only a small part of the low moor peat, tended to be low with the low moisture and high with the higher moisture, whereas the alcohol-soluble substances tended to increase with conditions more favorable to decomposition. The hemicelluloses did not change appreciably in concentration, but the concentration of the lignins and proteins tended to increase. The general conclusion from these analyses is that the composition of the peat was in a condition of relative equilibrium, in

TABLE 7

Alkali solubility of peat materials

Per cent of dry peat

DEPTH	KEPT AT A MOISTURE CONTENT OF	SOLUBLE IN COLD 2 PER CENT NH_4OH	SOLUBLE IN HOT 4 PER CENT NaOH AND PRECIPITATED BY HCl	TOTAL ALKALI SOLUBLE
cm.	*per cent*			
0–30	Control	30.0	24.3	54.3
0–30	83.8	22.4	35.8	58.2
0–30	79.9	19.8	35.7	55.5
0–30	71.3	39.5	26.1	65.6
0–30	52.8	37.6	25.6	63.2
0–30	33.3	25.6	34.2	59.8
30–60	Control	15.8	35.6	51.4
30–60	89.8	11.8	40.0	51.8
30–60	73.5	21.7	34.8	56.5
30–60	69.5	21.0	34.6	55.6
30–60	58.8	22.9	31.1	54.0
60–75	Control	25.6	26.4	52.0
60–75	90.9	9.5	47.8	57.3
60–75	81.3	35.4	30.6	66.0
60–75	72.8	28.8	31.2	60.0
60–75	63.0	15.2	38.3	53.5

which the various constituents decompose alike; here again the decomposition of the peat is quite different from that of fresh plant residues.

In order to determine whether any differences are found in the so-called "humus" fractions of the peat, the fresh and decomposed samples of peat were first treated with dilute HCl to remove the bases. Definite amounts of the samples thus treated were washed and dried and extracted with cold 2 per cent NH_4OH solution. The extract was filtered off, evaporated, dried to constant weight, and ignited. The residues from the ammonia extraction were treated with 4 per cent NaOH solution at 15 pounds pressure for 1 hour; the filtered extracts were neutralized with HCl and the precipitates formed were removed, washed, dried, and weighed (table 7). The results show that under conditions

of optimum decomposition there is an increase in the alkali-soluble fractions, especially the one soluble in cold dilute ammonium hydroxide (matière noire of Grandeau). It is doubtful whether this is merely a stage in the decomposition; it is probably more a result of the decomposition of the resistant lignin and protein complexes, as found to be the case in the decomposition of manure composts (1).

One need not, however, expect that the results obtained from the foregoing investigations on the decomposition of the lowmoor peat from Florida would hold exactly true for other peats or even for other lowmoor peats. This can well be illustrated by reporting the results of another experiment on the influence of drying of peat upon its decomposition; for this purpose, a lowmoor (*Carex*) peat from Newton, N. J., was used (3). The previous peat, from Florida, was taken fresh from the field, so that it had very little chance to decompose aerobically, except in the surface layer, before the experiment was

TABLE 8

Influence of moisture upon the rapidity of decomposition of a lowmoor peat from Newton, New Jersey

On basis of 20 gm. of dry peat

TREATMENT OF PEAT	MOISTURE CONTENT	CO_2 LIBERATED IN 21 DAYS	NITROGEN LIBERATED IN 21 DAYS		
			NH_3-N	NO_3-N	Total N
	per cent	*mgm. C*	*mgm.*	*mgm.*	*mgm.*
Untreated, 60 gm.	66.6	16.4	1.6	8.2	9.8
Air-dried,* 23 gm., + 20 cc. water	53.5	115.1	25.1	3.8	28.9
Air-dried,* 23 gm., + 37 cc. water†	66.7	41.9	9.6	5.6	15.2
Air-dry peat, 11.5 gm., + 30 gm. moist peat	51.8	109.4	22.4	4.2	26.6

* Only 15 per cent moisture left.

† Saturated with water, not well absorbed; anaerobic environment.

started. The Newton peat was well decomposed in a pile, probably for a year or so, before it was used for the following experiment. The peat, when taken from the pile, contained 33.3 per cent dry matter. Sixty-gram protions of this peat were used for the decomposition studies. Some of the portions were left untreated, whereas others were thoroughly air-dried in the laboratory, so that the moisture was reduced to 15 per cent of the peat. When the same amount of distilled water was added to replace the loss, it was not well absorbed and the peat looked saturated with water. Only enough water was taken up by the peat to bring it to 53.5 per cent moisture.

The results given in table 8 show that the air-dried peat remoistened decomposed much more rapidly than the original peat remaining moist. However, the rate of decomposition of the air-dried peat was markedly reduced if an excess of water was used. The ratio between the carbon liberated as CO_2 and the nitrogen liberated as ammonia was considerably narrower in the case of this peat than in the case of the Florida peat. This is due not so much to the

difference in the chemical composition of the two peats, as to the fact that the Newton peat was allowed to compost for a long time, exposed to air and rainfall, whereas the Florida peat was freshly taken from the bog.

SUMMARY

1. The moisture content of peat was found to have a marked influence upon the rate of its decomposition.

2. Under optimum moisture conditions, about 15 per cent of the total dry material of lowmoor peat was decomposed in 18 months, as indicated by the volatile products liberated.

3. By controlling the amount of water in the peat, one can almost at will control the speed of peat decomposition.

4. The optimum moisture content for the decomposition of a lowmoor peat was found to be at 50 to 80 per cent of the total moist peat; above and below that optimum, the rate of peat decomposition rapidly diminishes.

5. The lowmoor peat used in these experiments was in a condition of chemical equilibrium, and decomposed as a whole, without any chemical complexes disappearing more rapidly than others. However, there was a certain tendency for a somewhat more rapid decomposition of the non-nitrogenous than the nitrogenous complexes, which led to an increase in the nitrogen content of the residual peat.

6. In the process of decomposition of a lowmoor peat, there was a parallel liberation of carbon as CO_2 and of nitrogen as ammonia, with an average ratio of C/N liberated as 20:1. This is somewhat wider than the C/N ratio of the peat itself, which is about 18:1, and tends to explain the increasing nitrogen content of the peat as a result of decomposition.

7. As a result of the decomposition of the peat there was an increase in the complexes soluble in dilute alkali solutions.

8. Drying of peat and then remoistening it greatly stimulated its decomposition.

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THE INFLUENCE OF TEMPERATURE ON THE NITRATE CONTENT OF SOIL IN THE PRESENCE OF DECOMPOSING CELLULOSE[1]

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It is commonly known that the decomposition of cellulose in soil results in the depletion of soil nitrates. In certain sections of the country farmers have learned that in order to avoid impoverishment of the soil they must remove straw from the field instead of plowing the straw into the soil. The microorganisms engaged in decomposing cellulose utilize the soil nitrate. Consequently, unless a soil is rich in organic nitrogen which can be converted readily into nitrate, nitrogen starvation of crops results. In addition to an adequate supply of organic nitrogen in the soil, suitable conditions of temperature, moisture, and soil reaction must obtain if nitrification is to proceed satisfactorily.

Among the investigations dealing with the depletion of soil nitrates as a result of cellulose decomposition in soil, those of Sievers and Holtz (13), Collison and Conn (3), and Allison (1) may be mentioned. These publications contain reviews of the literature on the subject.

Most of the studies concerning the effect of temperature upon the nitrate content of the soil during the process of cellulose decomposition have been devoted to the influence of seasons and have been carried out in the field. Batham and Nigam (2), Jenny (6), Owen and Denson (10), and Panganiban (11) have reported studies along this line. Russell, Jones, and Bahrt (12) investigated the influence of varying temperatures on the process of nitrification, but did not consider the effect of cellulose.

The present study was suggested by results obtained by Jones (7) in the use of paper pots for the growth of plants in the greenhouse. The foliage of the plants turned yellow. When some of the plants were removed from the pots and examined it was found that the roots had grown toward the periphery of the ball of soil and were in contact with the water-soaked paper of the pots. This led to the assumption that the microörganisms which were attacking the paper of the pots had utilized the nitrate of the soil. When readily available nitrogen was supplied to the plants they regained their normal color, demonstrating that the yellowing was due to nitrogen starvation.

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It was further observed that the degree and rapidity of the yellowing of the plants seemed to vary with seasons. This suggested the possibility that some seasonal factor may have been responsible for the variation. Since the amount of moisture added to the pots was uniform, it appeared probable that temperature was the factor which influenced the degree and severity of the yellowing of the plant foliage.

The experiments reported in this paper were planned to investigate the influence of temperature on the depletion of soil nitrates as a consequence of cellulose decomposition in the soil.

EXPERIMENTAL

The experimental work was divided into two parts. Part I consisted of a series of four experiments in which fallow soil was employed. Periodic nitrate

TABLE 1

The plan of the experiments

	EXPERIMENT I	EXPERIMENT II	EXPERIMENT III	EXPERIMENT IV
Soil treatment	Soil untreated	Soil untreated Soil + $CaCO_3$ Soil + $CaSO_4$	Soil untreated	Soil untreated Soil + $CaCO_3$
	Soil + cellulose	Soil + cellulose Soil + cellulose + $CaCO_3$ Soil + cellulose + $CaSO_4$	Soil + cellulose	Soil + cellulose Soil + cellulose + $CaCO_3$
Incubating temperature, °C.	10, 15, 20, 25, 30, 35	15, 25, 35	10, 15, 20, 25, 30, 35	10, 15, 20, 25, 30, 35
Time of incubation	7 weeks	7 weeks	7 weeks	7 weeks

determinations were made from the soil of the various pots to indicate the influence of decomposing cellulose on the nitrate content of the soil. Part II consisted of four experiments in which the soil employed, its treatment, and the incubating temperatures were identical in each instance with those of the first section. Tomato plants were grown in the pots of the second section, the dry weight of the plants at the end of the growing period being taken as an indication of the nitrate available in the soil. The two parts of the experimental work are considered separately in the following pages. The plan of the experiments is shown in table 1.

The soil employed in this investigation was a light, sandy loam common in the valley of the Connecticut River. The compost used in the third experiment was made from the same type of soil. All soil was air dried and screened to give it uniformity and to remove stones, roots, and other debris. Cellulose and other materials were carefully mixed with the soil before it was put into the

pots. Into each pot 5½ kgm. of soil were put. Water was added and maintained at 60 per cent of saturation. Metal pots were used which had no provision for drainage. Soil temperature was maintained by setting the pots into a constant soil-temperature apparatus which is illustrated in plate 1.

Incubation was carried on for 7 weeks for each experiment, samples of the soil for nitrate determination being taken each week. The samples were of sufficient quantity to yield 100 gm. of air-dried soil from each pot. The soil was dried at about 25°C., and screened through a 20-mesh sieve. The nitrate determinations were made by reduction method mentioned by Fred and Waksman (4), which employs Devarda's Alloy as a reducing agent. The technic is as follows:

> Extract 100 gm. of soil with 500 cc. of distilled water. (The extraction was accomplished by vigorous shaking for 10 minutes in a mechanical shaker.) Filter through filter paper. Collect 250 cc. of the filtrate in a Kjeldahl flask. Add 5 cc. of a 50 per cent solution of sodium hydroxide and distil until 150 cc. have been driven over. The distillate contains the free ammonia of the soil and is discarded. Make the contents of the flask up to the original volume with distilled water and allow to cool to room temperature. Add 2 gm. of Devarda's alloy and distil slowly until 150 cc. have been driven over into a measured quantity of $N/14$ sulfuric acid. After distillation is completed, titrate the sulfuric acid with $N/14$ sodium hydroxide, using Congo red as an indicator. The results are obtained as milligrams of nitrogen in 50 gm. of soil, and are calculated to parts per million of nitrate.

The reduction method was chosen in preference to a colorimetric method because with the former a direct nitrate determination can be made on a much larger quantity of soil than with the latter. For purposes of comparison a number of the samples were also analyzed for nitrate content with a colorimetric method which employs phenoldisulfonic acid. The results obtained with the reduction method compared satisfactorily with those obtained with the colorimetric method, the former being somewhat more uniform for a series of samples from the same soil.

In experiments in which lime was employed the pH value of the soil was determined electrometrically at the end of the incubation period, a quinhydrone electrode being used.

PART I. INFLUENCE OF ADDED CELLULOSE ON SOIL NITRATE AS INDICATED BY THE NITRATE DETERMINATIONS FROM THE SOILS

Experiment 1

The soil chosen for this experiment was low in nitrate as well as in total nitrogen. There were 50 ppm. of nitrates and less than 1 mgm. of total nitrogen per gram of soil. The soil had not been previously limed nor treated with fertilizer. Six temperatures were used for incubation, from 10° to 35°C. inclusive at intervals of 5°. At each temperature, pots containing soil without cellulose and pots containing soil plus 1 per cent by weight of cellulose in the form of finely ground wheat straw were incubated for 7 weeks. The results of experiment 1 are shown in figure 1.

Throughout the experiment the nitrate content of the soil containing cellulose was definitely lower than that of the soil without cellulose. There was no recovery of the nitrate content in the soil containing cellulose at any time during the experiment. There is an apparent lack of uniformity in the results illustrated in figure 1. This may have been due to the necessity of drawing the graph on an exaggerated scale, because of the small amount of nitrates present, in order to show the relationships between soil with and soil without cellulose at the different temperatures.

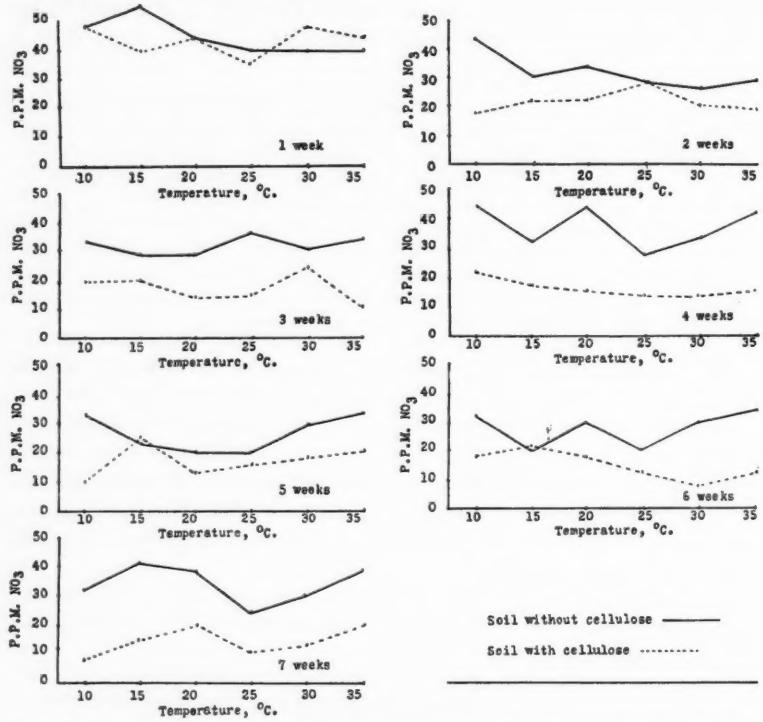

FIG. 1. NITRATE AT DIFFERENT TEMPERATURES IN A NITROGEN-POOR SOIL WITHOUT AND WITH ADDED CELLULOSE

The nitrate level of the soil in all of the pots was considerably lower than the initial nitrate content of the soil employed. This indicated some loss of nitrate in all of the pots, the loss in the presence of cellulose being greater than that in the absence of cellulose. The loss of nitrate could not have been due to leaching because the pots were not drained.

Experiment 2

Since the soil employed in experiment 1 had never been limed it was decided to investigate the influence of calcium salts on the nitrate content of soil in

the presence and absence of cellulose. The nitrate content of the soil employed was 70 p.p.m. Pots were prepared according to the following plan: soil untreated, soil plus calcium carbonate, soil plus calcium sulfate, soil plus cellulose, soil plus cellulose and calcium carbonate, soil plus cellulose and calcium sulfate. The calcium salts were added at the rate of 5 gm. per kilogram of soil. One per cent of finely ground wheat straw was added for cellu-

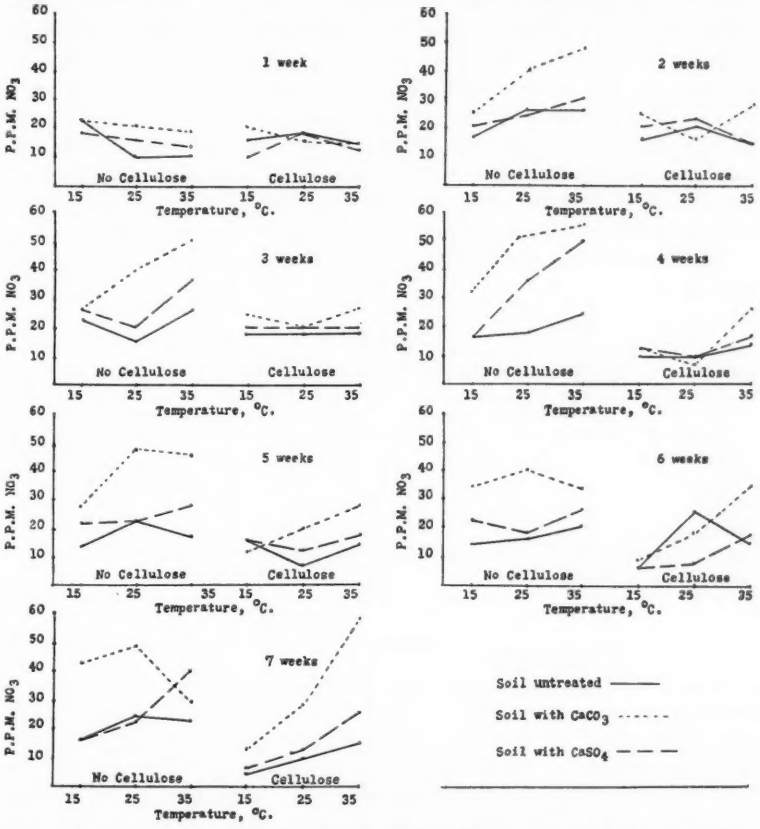

FIG. 2. NITRATE AT DIFFERENT TEMPERATURES IN A NITROGEN-POOR SOIL WITHOUT AND WITH ADDED CELLULOSE, AND TREATED WITH $CaCO_3$ OR WITH $CaSO_4$

lose. Pots representing each of the treatments were incubated at each of three temperatures, 15°, 25°, and 35°C. Limitation of equipment made it impossible to use the entire temperature range employed in the first experiment, so temperatures were chosen to represent the low, median, and high of the temperature range previously employed. The incubation period was 7 weeks as before. The results of experiment 2 are shown in figure 2.

In soil with and in soil without cellulose, neither of which contained added calcium, the observations made agreed with those from experiment 1. In the latter half of the experiment at 20°C. and above, there was less nitrate depletion in soil containing calcium carbonate plus cellulose than in soil containing cellulose and no calcium carbonate. The greatest concentration of nitrate was observed in soil at 35°C. At 15°C. there was some depletion of the soil nitrate in soil containing calcium carbonate and no cellulose. In the same soil, during the latter half of the experiment, and at 25° and 35°C., the nitrate content of soil remained nearly equal to the initial nitrate content of the soil. In general it may be stated that calcium carbonate was instrumental in maintaining a higher nitrate content in soil in both the presence and absence of cellulose, than was apparent in soil with parallel treatment except that added calcium carbonate was lacking. The nitrate content of soil containing calcium carbonate and cellulose remained lower than that of soil containing calcium carbonate and no cellulose. Calcium sulfate had no significant effect.

The influence of the added calcium salts on the pH values of the soil is shown in table 2.

TABLE 2

The pH value of soils of experiment 2 after 7 weeks' incubation

TREATMENT OF SOIL	pH AT 15°C.	pH at 25°C.	pH at 35°C.
No treatment	6.1	5.9	5.7
$CaCO_3$	6.6	6.4	6.2
$CaSO_4$	6.1	6.0	5.6
Cellulose	6.1	6.0	6.0
Cellulose and $CaCO_3$	6.0	6.5	6.5
Cellulose and $CaSO_4$	5.7	6.0	5.6

Experiment 3

Since the soil employed in experiment 1 was low in nitrogen and total nitrates, a well-seasoned compost soil was chosen for the third experiment in order that results obtained from soils of high and low nitrate and nitrogen content might be compared. The compost had been made by combining manure with soil of the same quality as that used in the first two experiments. No lime had been applied to the soil. The experiment was prepared in the same manner as experiment 1, except that finely cut paper toweling, instead of straw, was used for cellulose. Straw appeared to be better adapted than paper for use with poor soil because straw contained some nitrogen which could be converted into available nitrogen for the use of the cellulose decomposing organisms. The compost soil naturally had no need for additional nitrogen, so paper was substituted for the straw. The incubating temperatures were the same as those employed in the first experiment, 10° to 35°C. inclusive at intervals of 5°. The incubation period was 7 weeks. The results of the experiment are shown in figure 3.

In this experiment at temperatures below 25°C., the nitrate content of soil containing cellulose was definitely less than that of soil without cellulose. This agrees with the results obtained in the first experiment. During the latter half of the third experiment at 25°C. and above, the nitrate content of soil containing cellulose approached, and in some cases was equal to, the nitrate content of soil without cellulose. This observation indicated the ability of a soil rich in organic nitrogen to produce nitrate in sufficient quantity to replace that lost as a result of cellulose decomposition. It might be argued that the higher

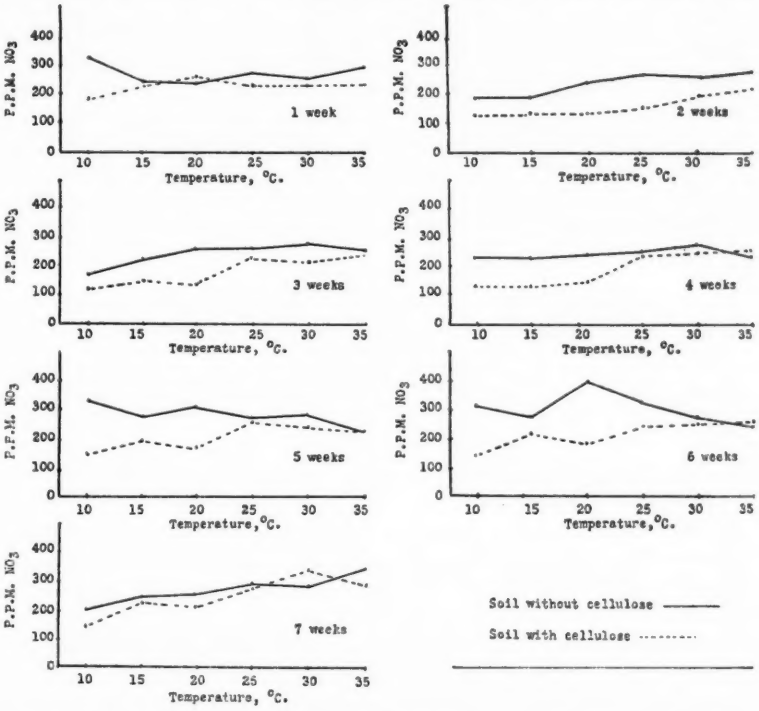

FIG. 3. NITRATE AT DIFFERENT TEMPERATURES IN A COMPOST SOIL WITHOUT AND WITH ADDED CELLULOSE

nitrate content observed under the conditions mentioned may have been due to a failure of the microörganisms to utilize the nitrate of the soil, rather than to increased nitrification. A comparison of the findings of experiment 1 with those of experiment 3 seem to indicate that active nitrate depletion had taken place and that the nitrate lost had been replaced. Certainly nitrate depletion was indicated in experiment 1.

In the absence of cellulose the greatest concentration of nitrate was observed at 20° to 30° inclusive. When cellulose was present the maximum amount of

nitrate was observed at 25° to 35°C. inclusive. During the latter half of the experiment at 25°C. and above, the nitrate content of soil with and of soil without cellulose exceeded the initial nitrate content of the soil (220 p.p.m.).

The most important observation in this experiment was the ability of soil rich in organic nitrogen to maintain a high nitrate content in the presence of decomposing cellulose, particularly at higher temperatures and during the latter half of the experiment.

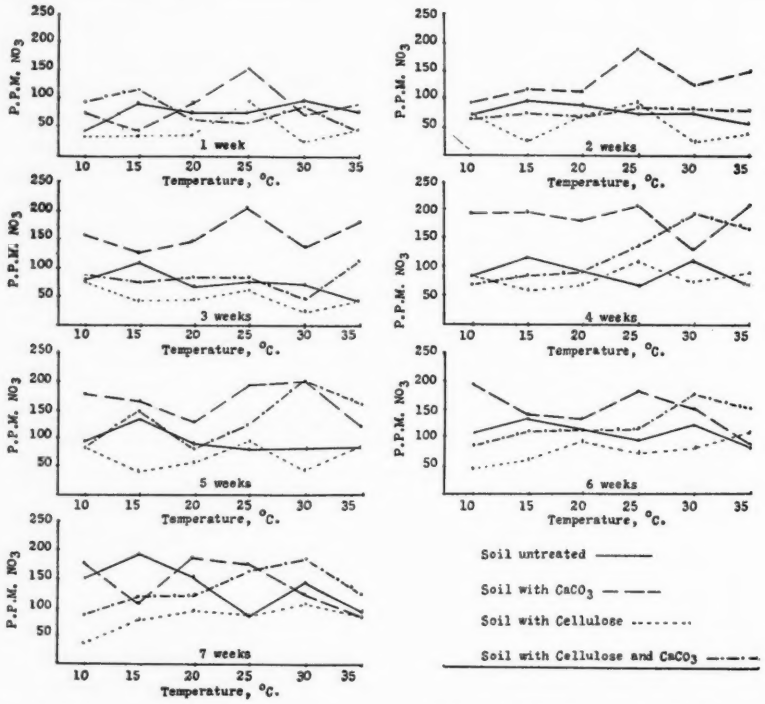

FIG. 4. NITRATE AT DIFFERENT TEMPERATURES IN A SOIL OF MEDIUM NITROGEN CONTENT, WITHOUT AND WITH ADDED CELLULOSE, AND TREATED WITH $CaCO_3$

Experiment 4

In order to study the influence of added calcium salts under the conditions of experiment 3, an experiment was planned similar to experiment 2. Since the compost employed in the third experiment was not available, a soil was chosen containing 95 p.p.m. of nitrates and 1.5 mgm. of total nitrogen per gram of soil. Wheat straw was again employed for cellulose. It was thought that the nitrogen of the soil together with that of the straw should furnish sufficient nitrogen to indicate any ability the soil might have to produce nitrates under the conditions of the experiment. This proved to be true.

The experiment was set up as follows: untreated soil, soil plus calcium carbonate, soil plus cellulose, soil plus cellulose and calcium carbonate. Calcium carbonate was added at the rate of 5 gm. per kilogram of soil. Calcium sulfate was not employed because it had had little apparent influence when used in the second experiment, and by its elimination it was possible to employ the same temperature range as that used in the third experiment. One per cent by weight of cellulose was used. Pots representing each of the aforementioned treatments were incubated at each of six temperatures, 10° to 35°C. inclusive, at intervals of 5°. Incubation was continued for 7 weeks. The results of experiment 4 are shown in figure 4.

In pots without calcium carbonate the observations made in the third experiment were repeated but on a less extensive scale because of the lower initial nitrate and nitrogen content of the soil. The addition of calcium carbonate to soil without cellulose resulted in an increase of nitrate above the initial nitrate content of the soil at and above 20°C. The nitrate content of the soil was slightly less at 35° than at 20° to 30°C. inclusive. The addition of calcium carbonate to soil containing cellulose appeared to stimulate the increase

TABLE 3

The pH value of soils of experiment 4 after 7 weeks of incubation

TREATMENT OF SOIL	pH AT 10°C.	pH AT 15°C.	pH AT 20°C.	pH AT 25°C.	pH AT 30°C.	pH AT 35°C.
No treatment	6.2	5.8	5.9	5.8	6.0	6.1
$CaCO_3$	6.9	6.7	6.9	6.8	6.9	6.9
Cellulose	5.8	6.4	5.7	5.8	5.8	6.0
Cellulose and $CaCO_3$	6.8	7.0	6.8	6.7	6.8	6.9

of nitrate during the latter half of the experiment at 25°C. and above. The nitrate concentrations of soil containing calcium carbonate, both with and without cellulose, were practically equal at 25°C. and above, during the latter half of the experiment. These concentrations were considerably above the initial nitrate concentration of the soil. Apparently 35° was a less favorable temperature than 25° and 30°C. for a high nitrate concentration in the soils.

The principal observation from this experiment was that at 25°C. and above the addition of calcium carbonate to soil with or without added cellulose resulted in an increase in the amount of nitrate in the soil. The influence of calcium carbonate on the pH values of the soil is shown in table 3.

PART II. INFLUENCE OF ADDED CELLULOSE ON SOIL NITRATE AS INDICATED BY THE DRY WEIGHT OF TOMATO PLANTS

The experiments of part I with soils receiving applications of cellulose, calcium carbonate, and calcium sulfate were carried on in containers from which samples were taken each week for nitrate determinations. It was not possible to grow plants in these containers as the sampling procedure would have muti-

lated the root systems. Consequently a parallel series of soils containing plants constituted one-half of each experiment. This series constitutes part II of the study. The soil in the containers with the plants was not disturbed. It is assumed that the processes taking place in the fallow containers were duplicated very closely in the parallel series containing plants.

TABLE 4

Relative dry-weight yields of tomato plants from soils of low and high initial nitrate content at different temperatures. With and without cellulose, and with and without calcium carbonate and calcium sulfate

TEMPERATURE, °C.	INITIAL NO_3 CONTENT OF SOIL	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		EXPERIMENT IV	
		54 p.p.m.		70 p.p.m.		220 p.p.m.		95 p.p.m.	
	Chemical treatment	Relative values (100 = 11.0 gm.)*		Relative values (100 = 9.8 gm.)		Relative values (100 = 54.6 gm.)		Relative values (100 = 5.7 gm.)	
		Without cellulose	With cellulose	Without cellulose	With cellulose	Without cellulose	With cellulose	Without cellulose	With cellulose
10	None	17	15			3	3	23	9
	$CaCO_3$							19	9
15	None	32	17	28	27	71	56	26	28
	$CaCO_3$			54	16			37	39
	$CaSO_4$			27	15				
20	None	33	11			85	90	49	49
	$CaCO_3$							95	67
25	None	53	15	73	43	103	90	86	124
	$CaCO_3$			164	77			118	144
	$CaSO_4$			80	28				
30	None	81	35			109	109	118	142
	$CaCO_3$							141	218
35	None	100	40	100	97	100	113	100	148
	$CaCO_3$			186	169			137	167
	$CaSO_4$			85	38				

*The absolute weight in grams of the plant may be obtained by multiplying the weight at the head of the tabulation of each experiment by the relative value divided by 100.

The tomato plant was used as an indicator plant. Three plants were set in each container. Because of the upright growth habit of the tomato plant there was little danger of one set of plants shading an adjoining set. The experiments were set up at the same time as those of part I. They were continued for 7 weeks and concluded on the day of the seventh sampling of the soils from the pots of part I. At the conclusion of each experiment, the plants were measured, cut at the surface of the soil, dried at 101°C., and weighed. Table 4

presents a composite tabulation of the weights from the four experiments. These weights are in terms of relative values with the weight of the plants in a non-cellulose soil at 35°C. equal to 100. In three of the four experiments, this temperature gave the highest yield in the absence of any soil treatment.

Cellulose mixed with a soil of low nitrate content considerably reduced the dry weight of the plants. This was particularly true at the temperature optimum for the growth of the tomato plant, which is close to 25°C., according to Jones, Johnson, and Dickson (8), and to Godfrey (5). However, if the soil had a very high level of fertility, as was the case in experiment 3, cellulose had practically no effect on the dry weight of the plants. The dry weight increased directly as the soil temperature was raised, and this increase in weight, in the soil without added cellulose, was uniformly paralleled in the series to which cellulose had been added. The nitrate content of this soil was markedly reduced by the added cellulose, but the low nitrate levels attained were still above the requirements of the plants in every case. The effect of cellulose on the yield of dry matter in experiment 4 presents a situation that must be interpreted on the basis of temperature effect on the plant and microbial activity in the soil. The initial nitrate content of this soil was neither high nor low and the crop employed was sensitive to the variations of the nitrate content as affected by temperature and the added cellulose and calcium carbonate. It seems quite apparent that nitrification was active at temperatures of 25°, 30°, 35°C., and this nitrification was more intense in the soils to which cellulose had been added. At temperatures of 15° and 20°C., there was no effect due to added cellulose, but at the lowest temperature, 10°C., the cellulose materially reduced the nitrate content of the soil, which was reflected in a lower crop yield.

The effect of calcium carbonate on dry weight of the plants was more apparent at the higher temperatures than at the lower temperatures. In a soil with an initial low nitrate content (experiment 2) calcium carbonate stimulated nitrification in both the presence and absence of added cellulose, but the increase of nitrates was greater in the soils to which no cellulose had been added. In soil of fair nitrate content (experiment 4) calcium carbonate was ineffective at the lower temperatures of 10° and 15°C.; but at temperatures of 20°C. and above, the dry weights of the plants indicated increased nitrification. In experiment 4 the nitrification was intensified by the presence of added cellulose. This was not the case in experiment 2 which employed a soil with a very low nitrogen content.

Calcium sulfate, added to the soil in an amount sufficient to give a calcium content equal to that in calcium carbonate, seemed to have a depressing effect on the yield of dry plant material, but this depressing effect is not correlated with any effect of calcium sulfate on the nitrate content of the soil. The calcium sulfate was used in experiment 2, and, as it did not prove to be a substitute for calcium carbonate, it was not used in the succeeding experiments. Calcium sulfate had a depressing effect in the soil to which cellulose had been added.

DISCUSSION

The results in the first experiment showing that there was less nitrate in soil containing cellulose than in soil without were to be expected and are in agreement with the experimental findings of other investigators and with agricultural practise. If the experimental results reported in this paper are to be significant, they should demonstrate conditions under which the nitrate content of soil can be maintained or increased in the presence of cellulose. The second, third, and fourth experiments appear to demonstrate such conditions.

In the second experiment there was less loss of nitrate, during the latter half of the experiment at 25°C. and above, from soil containing cellulose and calcium carbonate than from soil containing cellulose and no calcium carbonate. Under these conditions 35°C. appeared to be a more favorable temperature than 25°C. for increased nitrate. In the third experiment in which cellulose was added to soil rich in organic nitrogen there was sufficient nitrification at 25°C. and above to replace the nitrate lost as a result of cellulose decomposition. This was particularly true during the latter half of the experiment. In the fourth experiment the addition of calcium carbonate to soil with and to soil without cellulose resulted in a nitrate concentration in the soil under either condition which was greater than the initial nitrate concentration of the soil employed, and which was in contrast with the loss of nitrate in the same soil containing cellulose but no calcium carbonate.

The observation in the second and fourth experiments that the presence of calcium carbonate in soil results in increased nitrate content agrees with the statement of Noyes and Conner (9) who reported that calcium carbonate caused an increase in nitrification in soil.

Panganiban (11) and Russell, Jones and Bahrt (12) found the optimum temperature for nitrification to be 35°C. In the experiments reported in this paper 25° and 30°C. proved in most instances to be the more favorable temperatures for increase in nitrate.

Jenny (6) stated that it is easier to build up a soil in a warm climate than in a cooler one. No doubt this is true in general, but the first two experiments here reported indicate that it would not be advisable to plow straw into a poor soil regardless of the temperature. Not even the addition of lime would prevent serious nitrate depletion in such a soil.[4]

[4] Visual evidence was depended upon in determining the fact that the cellulose was decomposed in the soils during the course of the experiments. At the beginning of each experiment the cellulose could be seen plainly in any sample of the soil. At the end of each experiment practically no cellulose was visible in soil held at the higher temperatures, and very little in soil held at 15°C. and below. This was equally true of straw and paper.

In order to investigate the influence of temperature on the rate of cellulose decomposition the following experiment was planned: Culture tubes were prepared containing medium no. 85 from the Fred and Waksman (4) laboratory manual. Strips of filter paper were inserted into the tubes so that a part of the paper in each tube was immersed in the fluid medium.

SUMMARY

The influence of temperature on the nitrate content of soil in the presence of decomposing cellulose has been investigated. Soils low and soils high in organic nitrogen and with and without added cellulose were maintained at temperatures of from 10° to 35°C. for a period of 7 weeks. A series of fallow soils was paralleled by a series of soils from the same sources which were identically treated and which contained tomato plants. Certain of the soils of these two series were treated with calcium carbonate or with calcium sulfate.

In soil which was low in organic nitrogen the addition of cellulose resulted in a depletion of nitrate at all temperatures. Calcium carbonate added to this soil in the presence of added cellulose reduced the nitrate depletion during the latter half of the time period at 20°C. and above. Calcium sulfate did not prove to be a substitute for calcium carbonate.

In soil which was rich in organic nitrogen, added cellulose resulted in nitrate depletion at all temperatures during the first half of the time period. During the latter half of the time period at temperatures of 25°C. and above the nitrate content of the soil containing added cellulose showed a definite tendency to equal the nitrate content of the same soil without added cellulose. The addition of calcium carbonate to this soil with and without added cellulose stimulated nitrification.

The crop response as indicated by the dry weights of the tomato plants was in close agreement with what would be expected from the nitrate determinations made from the fallow series. The dry weight of the plants was considerably reduced by the addition of cellulose to a soil low in nitrogen. In a soil high in organic nitrogen the addition of cellulose reduced the nitrate content, but not to a level that impoverished the soil for the requirements of the crop. Calcium carbonate stimulated greater growth of the plants at the higher temperatures in both the presence and absence of added cellulose. Calcium sulfate seemed to have a depressing effect on the yield of dry plant material.

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The tubes were sterilized and inoculated with 1 gm. each of soil known to possess active cellulose-decomposing properties. The tubes were buried in the soil of the various pots to provide incubation at the different temperatures employed in the experiments reported. The tubes were bent so that most of the length was horizontal at a depth of 2 inches below the surface of the soil, with only the plugged end of each tube protruding. After 10 days' incubation the tubes were examined. Cellulose decomposition was indicated by decomposition of the paper at the surface of the fluid in the tubes. The cellulose-decomposing activity appeared to be equally vigorous at all temperatures except at 7° and 10°C., where decomposition was evident but retarded.

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PLATE 1

CONSTANT SOIL TEMPERATURE APPARATUS

FIG. 1. Complete apparatus.

FIG. 2. A unit of the apparatus. The bimetallic thermo-regulator, 1, controls the relay, 2, which cuts in or out the current to the strip heaters, 3. The strip heaters are inserted in the oven, 4, which is a cell extending into the tank, 10. The thermometer, 5, indicates the temperature of the water bath. Cooling water is obtained from the main at 7 and enters a pipe at 6 which leads to the bottom of the tank where it creates circulation currents and overflows at 8. The soil containers number four in each unit and may be easily removed as is shown at 9.

FIG. 1

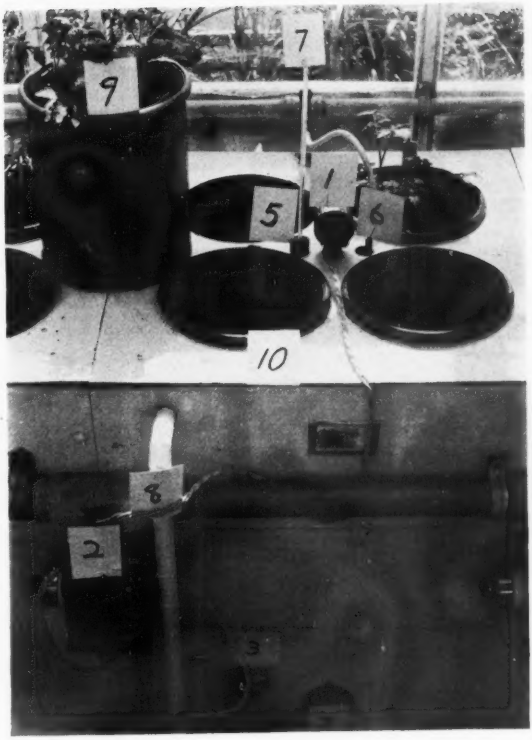

FIG. 2

CAUSES OF LOW NITRIFICATION CAPACITY OF CERTAIN SOILS[1]

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In connection with some studies of nitrification in Texas soils, it was found that a number of soils had no power to nitrify ammonium sulfate. Small amounts of nitrates would be produced from the organic matter of the soil, but where ammonium sulfate was added, the quantity of nitrates would frequently be less than when no addition was made. Stevens and Withers (8) reported that a number of North Carolina soils failed to nitrify organic nitrogen, but offered no explanation. Lipman (6), Sackett (7), Kelly (5), and perhaps others reported soils which did not nitrify. Gainey (4) claimed that the failure to obtain nitrification was due to the particular experimental conditions and not to the entire absence of active organisms. In the course of our work a number of soils were found which, although they usually produced some nitrates from the soil nitrogen, did not nitrify ammonium sulfate. Because it seemed a matter of some importance to find out why these soils failed to nitrify, extensive experiments were conducted for this purpose.

METHOD OF WORK

The method used was similar to that usually followed (10): 200 gm. of soil was mixed with inoculating liquid, 10 cc. of ammonium sulfate solution containing 0.1 gm. nitrogen, water equal to 50 per cent of the water capacity of the soil, placed in a 300-cc. tall Pyrex beaker, weighed and incubated at 35°C. Another portion received inoculating liquid and water only. The water lost by evaporation was replaced twice a week. At the end of 28 days, nitrates and nitrites were estimated by the methods already described (3). This general method was modified according to the conditions being studied.

EFFECT OF BACTERIA AND CALCIUM CARBONATE

After a considerable amount of preliminary work, which will not be discussed, we found that, by additions of portions of actively nitrifying cultures, of calcium carbonate, or of both nitrifying cultures and calcium carbonate, ammonium sulfate could be made to nitrify in soils which otherwise did not oxidize it. A large number of soils were found which did not nitrify ammonium sulfate under the usual conditions, but more than 90 per cent of these soils

[1] Technical Contribution No. 207, Texas Agricultural Experiment Station, presented at the New Orleans meeting of the American Chemical Society, March 30, 1932, and revised.

had a high nitrifying power when inoculated with nitrifying organisms, or when they received calcium carbonate, or both.

Table 1 contains some of the results which show this to be the case. The first soil, Susquehanna fine sandy loam, 0–7 inches, did not nitrify ammonium sulfate alone or with additions of calcium carbonate or of nitrifying soil. When both calcium carbonate and nitrifying soil were added, it nitrified a high percentage of the nitrogen in the ammonium sulfate.

TABLE 1

Effect on nitric nitrogen produced of addition of cultures and calcium carbonate to soils low in nitrifying power

ADDITION TO 200 GM. SOIL	NITRIC NITROGEN (P.P.M.) PRODUCED IN									
	35172 Susquehanna fine sandy loam, 0–7″	35170 Crockett fine sandy loam, 0–7″	31889 Webb fine sandy loam, 7–19″	32645 Lake Charles clay loam, 7–19″	32648 Lake Charles very fine sandy loam, 13–22″	33128 Garner clay, 0–7″	33130 Bowie fine sandy loam, 3–7″	33135 Susquehanna fine sandy loam, 0–7″	33137 Wilson clay, 0–7″	33712 Houston black clay, 18–24″
None	18	37	20	7	0	0	0	60	22	23
Ammonium sulfate (0.1 gm. N)	14	49	30	21	2	0	0	92	19	41
Calcium carbonate (2 gm.)	31	49	44	1	0	10	55	110	44	...
Calcium carbonate and ammonium sulfate	37	400	47	1	0	33	61	172	53	...
Nitrifying soil (10 gm.)	42	...	86	...	34	115	65	92	40	68
Nitrifying soil and ammonium sulfate	70	...	550	156	294	430	410	128	53	340
Nitrifying soil and calcium carbonate	44	...	...	512	40	160	...	108	120	...
Nitrifying soil, calcium carbonate and ammonium sulfate	320	...	...	...	370	600	...	525	360	...
Nitrifying soil sterilized	...	...	58	0	...	28	...	525	20	54
Nitrifying soil sterilized and ammonium sulfate	...	...	58	0	...	30	...	215	32	62

The second soil, Crockett fine sandy loam, 0–7 inches, did not nitrify alone but nitrified when calcium carbonate was added. Although it is well known that calcium carbonate would accelerate nitrification (10), it has not been recognized that calcium carbonate would cause soils to nitrify which otherwise did not nitrify ammonium sulfate at all.

The third soil, Webb fine sandy loam, subsoil 7–19 inches, had little nitrifying power alone or with calcium carbonate added, but when nitrifying soil was added, it developed a high nitrifying power. Results with other soils representing the various groups are given in table 1. Nitrification in all these soils

originally was low, but was decidedly increased by the nitrifying cultures or the calcium carbonate.

Of the large number of soils studied, 53 are classified, as shown in table 2. One-third of the 21 surface soils listed have a high nitrification power, while one-third have no nitrification power for ammonium sulfate. Approximately half of these are made to nitrify well by additions of calcium carbonate and one-half by additions of calcium carbonate together with soil containing actively nitrifying bacteria. Of the 32 subsoils, about 16 per cent have a high nitrifying power while 60 per cent have no nitrifying power for ammonium sulfate. Of those which have little or no nitrifying power, two, or about 10 per cent, are made to have high nitrification by bacteria alone, 1, or 5 per cent, by calcium carbonate, and 16, or 85 per cent, require both bacteria and calcium carbonate. The subsoils are much more frequently deficient in bacteria than

TABLE 2

Number of samples whose nitrification was increased by lime or bacteria compared to total number

	NUMBER OF SURFACE SOILS	NUMBER OF SUBSOILS
Nitrification capacity 70–100	7	5
Nitrification capacity 50–70	2	4
Nitrification capacity zero	(7)	(19)
Made high by bacteria alone	0	2
Made high by lime alone	4	1
Made high by lime and bacteria	3	16
Nitrification 1–20	(5)	(4)
Made high by bacteria alone	1	2
Made high by lime alone	2	2
Made high by lime and bacteria	2	0
Total number	21	32

the surface soils, but the number of surface soils deficient in nitrifying bacteria is comparatively large.

The nitrifying soil, of course, in addition to the nitrifying organisms, may carry soil materials which affect nitrification. The nitrifying organisms are usually the chief addition, as is shown when a portion of the nitrifying soil is sterilized, and added in the same way as the unsterilized culture. A few of such results are given in table 1. It is seen that the sterilized soil did not produce nitrification. Other experiments could be cited to the same effect. Of course, if the sterilized soil contained calcium carbonate in sufficient quantity, it might cause nitrification to take place in soils which need only calcium carbonate for this purpose, or which need calcium carbonate in addition to the bacteria.

The nitrifying soils used were portions of cultures obtained in previous nitrification experiments conducted by the methods previously described, and

contained some nitrates which of course can be allowed for by blank tests. If the soil needed calcium carbonate for nitrification, appreciable amounts of calcium carbonate in the nitrifying soil would affect the nitrification. In case it is desired to know whether the addition of nitrifying organisms produces nitrification without additions of calcium carbonate also, it is necessary to select cultures low in calcium carbonate for the inoculation. The effect of the other ingredients of the inoculating soil is being studied.

EFFECT OF INOCULATING LIQUID

It is customary to inoculate the soils used in nitrification experiments with liquids prepared from nitrifying soils (10). Inoculating liquid was likewise

TABLE 3

Effect of inoculating liquid upon production of nitrates, in parts per million of soil

LABORATORY NUMBER	SOIL TYPE, LOCATION AND DEPTH	NITRATES PRODUCED WITH AMMONIUM SULFATE		GAIN DUE TO INOCULATING LIQUID	NITRATES PRODUCED WITHOUT AMMONIUM SULFATE		GAIN DUE TO INOCULATING LIQUID
		Plus inoculating liquid	No inoculating liquid		Plus inoculating liquid	No inoculating liquid	
6731	Surface soil, Willacy Co., 0–6″	210	260	0	105	96	9
7355	Surface soil, Runnels Co., 0–6″	460	420	40	220	220	0
8815	Surface soil, Coryell Co., 0–4″	537	550	0	160	136	24
12533	Wilson loam, Ellis Co., 0–6″	600	600	0	152	160	0
12534	Wilson loam, Ellis Co., 6–18″	450	460	0	94	96	0
12576	Durant fine sandy loam, Ellis Co., 0–12″	400	390	10	122	112	10
29423	Upland blackland, Bell Co., 0–12″	331	275	56	47	51	0
29424	Bottomland, Bell Co., 0–12″	550	550	0	100	102	0
31884	Hidalgo clay loam, Frio Co., 0–7″	600	575	25	107	107	0
33702	Wilson clay, Collins Co., 0–7″	215	210	5	84	80	4
33709	Trinity clay, Collins Co., 7–24″	550	550	0	105	105	0
33710	Houston black clay, Collins Co., 0–7″	450	470	0	60	61	0

used in the work here reported and the discovery that the low nitrification in many soils was due to deficiency in nitrification bacteria was delayed because it was at first thought the organisms were being added in sufficient quantity by this inoculating liquid. After the great effect of additions of bacteria in cultures of nitrifying soils was discovered, it seemed desirable to ascertain the effect of the addition of inoculating liquid. The inoculating liquid was prepared by mixing 100 gm. of fresh field soil with 200 cc. of water; 10 cc. of the supernatant liquid was added to each culture when it was used. Air-dried soils which had been stored in the laboratory were used, with the results given in table 3.

It is apparent that the inoculating liquid had little or no effect upon the nitrification in these soils, and that the nitrification which occurred was brought

about by bacteria already present in the air-dried soil. If the inoculating liquid were thoroughly shaken and drawn off with the soil in suspension, it would no doubt carry some nitrifying organisms, but this amount would probably vary with the quantity of soil in suspension, and might be different for different portions of the same inoculating liquid. It appears that this method of adding nitrifying bacteria to the soil is not satisfactory.[2]

PERSISTENCE OF NITRIFYING BACTERIA IN DRY SOIL

The fact that the nitrification was caused by the bacteria in the dry soil is interesting in view of the fact some of these soils had been stored for long periods of time. Soil 6731 had been stored for 18 years; soil 7355, for 17 years; soil 8815, for 16 years; and soils 12533, 12534, and 12576, for 14 years. Although the samples were kept under laboratory, and not aseptic, conditions, and may have received small amounts of bacteria from the air during the period, the amount so received would hardly be sufficient to account for the quantities of nitrifying organisms present. Most of them must have persisted in the soil.

EFFECT OF NUMBER OF BACTERIA ADDED

It appeared from the results just presented that the results of a nitrification experiment might depend to a considerable extent upon the number of nitrifying bacteria present in the soil at the beginning of the experiment. It also seemed possible that different kinds of soil might give different results with the same number of added bacteria. Soils of low nitrifying power might require larger numbers of bacteria to produce the same amount of nitrates than soils with high nitrifying power.

For this reason, experiments were made by using different quantities of the same culture to supply different numbers of soil bacteria. In one series, different quantities of cultures from different soils were added to the same sterilized soil; in another series, the same culture was inoculated into different sterilized soils. Sterilization was effected by heating 2 hours at 140°C. in an electric oven.

Results on soils representative of the two series are shown in figures 1 and 2. The quantities of nitrates usually increased rapidly with the small additions of soil, but when more than 1 gm. of inoculant was added to 200 gm. of soil, the increase caused by the subsequent addition was low compared with the number of organisms added. The action of the bacteria followed the law of diminishing returns, in that each successive increment produced less increase in nitrification than the preceding addition. The nitrification was rarely in direct proportion to the quantity of inoculant added when it was small, that is, below 1 gm. to 200 gm. of soil, and never in proportion to the quantity added when the addition exceeded 1 gm. Hence it would not be correct to assume that the

[2] Since the foregoing was written, we have found that a suspension of 20 gm. of nitrifying soil in 500 cc. of water, added to a sterilized soil in quantities of 20 cc. or less, produces much greater amounts of nitrates than the corresponding quantity of the soil.

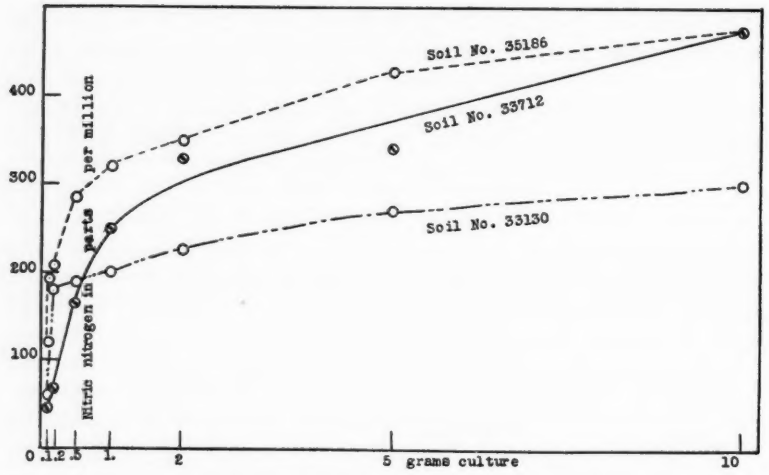

FIG. 1. RELATION OF THE NITRIC NITROGEN PRODUCED IN DIFFERENT STERILIZED SOILS TO THE QUANTITY OF THE SAME INOCULANT

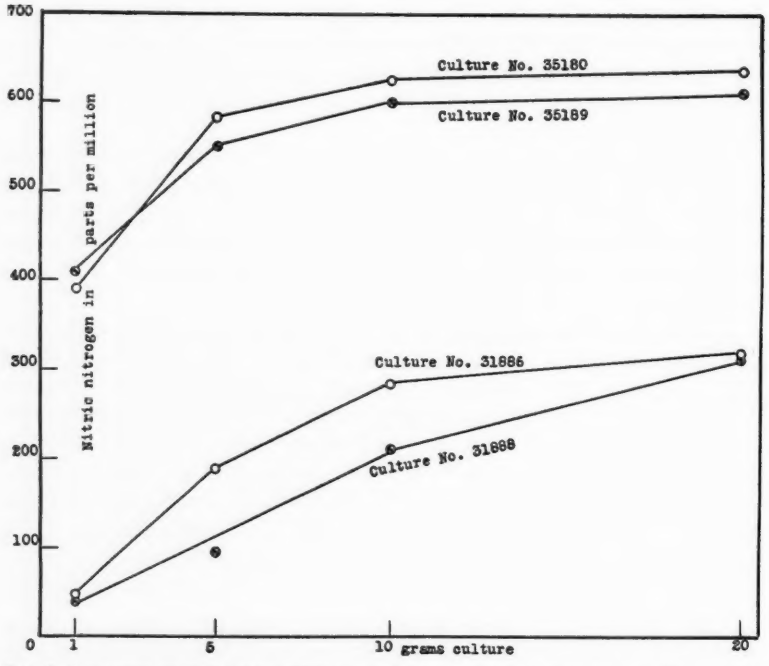

FIG. 2. RELATION OF THE NITRIC NITROGEN PRODUCED TO THE QUANTITY OF NITRIFYING CULTURES FROM DIFFERENT SOILS ADDED TO THE SAME STERILIZED SOIL

nitrates produced in an inoculated soil are in proportion to the number of bacteria present, or to use the quantity of nitrates as direct measure of the number of nitrifying bacteria in the inoculant.

In figure 1, the curves for the three soils are in two groups, parallel but widely separated, though the soils were sterilized and inoculated with the same culture. That is to say, the same quantities of nitrifying bacteria added to different soils produced different quantities of nitrates. This can, of course, be ascribed to differences in the chemical composition or physical properties of the two soils, and requires further study.

In figure 2, the same sterilized soil was inoculated with cultures from different soils. The four curves are in two groups similar in shape but they are located at different points on the scale. Cultures of soils 31886 and 31888 produce a maximum of 320 p.p.m. of nitric nitrogen, whereas cultures of soils 35180 and 35189 produce about 610. If the differences between culture 31886 and culture 35180 were due merely to a difference in the number of bacteria, we would expect these differences to be eliminated by the additions of increased quantities of culture 31886, and the same maximum to be attained. But increased additions of culture of soil 31886 had little effect, even though the quantity of nitrates produced was decidedly below the maximum produced by culture of soil 35180. Hence the differences must be due to differences in the nature of the bacterial complex in the two cultures, and not to differences in bacterial numbers. There must be decided differences in the bacterial complex in the two soils to produce such wide differences in the amounts of the end product. This difference might be due to different strains of nitrate and nitrite bacteria in the two cultures or to the presence of bacteria in culture 31886 which consume nitrates or nitrites as fast as they are formed after the high level is reached. It is a striking fact, as shown in figures 1 and 2, that similar results may be obtained with different cultures inoculated into the same sterilized soil, or by the same culture inoculated into different sterilized soils. In one case it is due to the bacterial complex, in the other case it is the character of the soil, which may act by modifying the bacterial complex.

The few other soils of high nitrification capacity which were tested responded to different amounts of inoculating culture in a similar way (fig. 1) to soil 35186. Soils of low nitrifying capacity, 33130 and 33712, at first produced less nitrate with the same number of organisms, but soil 33712 with 20 gm. inoculant produced as much nitrate as the soils of high nitrifying power, whereas soil 33130 did not. Other data regarding these two soils are given in table 1. Apparently, the same number of organisms may produce less nitrate in soils of low nitrifying capacity than in soils of high power, even though the difference may be small in some cases. This requires further study.

RELATION TO NITRITES

The foregoing discussion has related chiefly to nitrates. Nitrites, however, frequently occur in appreciable amounts in nitrification experiments. Since

we discovered this (1, 2), qualitative tests for nitrites have been made in all nitrification experiments, and quantitative estimations by methods already described (3), whenever appreciable amounts were found.

A number of soils which did not produce nitrates from ammonium sulfate alone or with calcium carbonate, produced large amounts of nitrites when calcium carbonate was added, showing that the nitrite organisms were present in sufficient number, even though the number of nitrate organisms was very low. Some of these soils are listed in table 4.

The sterilized soil inoculated with different amounts of cultures, as described in the preceding section, in many cases produced nitrites as well as nitrates. Some of these results are given in table 5. Generally the production of nitrites is highest with 0.1 gm. of inoculant to 200 gm. of sterilized soil, and decreases

TABLE 4

Production of nitrites induced by calcium carbonate

LABORATORY NUMBER	SOIL	NITROUS NITROGEN		NITRIC NITROGEN	
		Alone	With ammonium sulfate	Alone	With ammonium sulfate
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
33130	Bowie fine sandy loam, 3″–7″	0	168	55	61
33135	Susquehanna fine sandy loam, 7″–18″	0	106	19	24
35112	Miles fine sand, 0–7″	0	225	33	39
35113	Miles fine sand, 7″–24″	0	235	33	28
35114	Miles fine sand, 24″–36″	0	188	26	48
35168	Norfolk fine sand, 7″–36″	0	172	31	9
35169	Norfolk fine sand, 0–7″	0	245	38	33
35172	Susquehanna fine sandy loam, 0–7″	31	172	31	37
35184	Houston black clay, 24″–40″	4	69	9	17
35185	Tomlinson fine sandy loam, 21″–36″	0	288	17	17
35187	Tomlinson fine sandy loam, 7″–21″	0	152	21	40

with the increase in the amount of the inoculant, until practically no nitrite is produced with 2.0 gm. of inoculant. This would seem to indicate that either the nitrite organisms are more abundant in the inoculant than the nitrate organisms, or that they multiply more rapidly. Thus the low quantity of inoculant introduces sufficient numbers of nitrite organisms to produce appreciable amounts of nitrites, but not enough nitrate organisms to transform the nitrites into nitrates. Larger quantities of inoculant introduce more nitrate organisms, while the larger numbers of nitrite organisms so introduced are less effective. With 1 or 2 gm. of inoculant the nitrate organisms can transform all the nitrites produced into nitrates. This work also seems to indicate that the presence of high amounts of nitrites may be unfavorable to the growth of the nitrate organisms.

If we accept the general opinion that nitrogen must pass through the nitrous

stage before it becomes nitrates, the measure of nitrite production is the sum of the nitric and nitrous nitrogen. The production of nitrous nitrogen, measured in this way, is not in direct proportion to the number of organisms added in the inoculant. The first addition of 0.1 gm. of inoculant to 200 gm. of soil produces a high quantity of nitrites plus nitrates, whereas subsequent increments produce much smaller quantities. The effects of increments of inoculant are much less when nitrates and nitrites together are considered than when nitrates alone are used.

TABLE 5

Effect of amount of soil inoculant on nitrite formation (nitrogen parts per million)

LABORATORY NUMBER	KIND OF NITROGEN PRODUCED	QUANTITY OF INOCULANT USED							
		None	0.1 gm.	0.2 gm.	0.5 gm.	1.0 gm.	2.0 gm.	5.0 gm.	10.0 gm.
33128	Nitric N	0	21	24	84	117	220	287	293
	Nitrous N	0	105	51	25	40	0	0	0
33330	Nitric N	6	56	180	190	200	225	270	300
	Nitrous N	11	140	46	54	57	41	4	0
33711	Nitric N	8	218	243	225	360	400	440	487
	Nitrous N	0	74	60	106	0	0	0	0
33712	Nitric N	3	53	64	176	250	330	340	475
	Nitrous N	5	225	184	82	3	0	0	0
35179	Nitric N	14	26	76	120	240	337	390	430
	Nitrous N	112	180	164	160	80	11	0	0
35180	Nitric N	8	105	112	285	360	430	500	512
	Nitrous N	28	148	156	28	0	0	0	0
35186	Nitric N	8	110	205	290	320	350	425	475
	Nitrous N	2	84	10	13	7	2	0	0
35188	Nitric N	2	43	72	136	250	320	390	437
	Nitrous N	7	200	188	168	66	12	0	0

RELATION TO NITRIFICATION EXPERIMENTS

From the foregoing discussion, it is apparent that when two field soils are compared in nitrifying power, the results chiefly measure the number of organisms in the soil at the time the work was begun, though not accurately. The need for calcium carbonate may decrease the accuracy of this measure in some soils. The comparison does not measure the initial reaction, buffer content, or presence of neutralizing substances in the soil (9), for although these may have something to do with the number of organisms in the soil at a particular time, other factors also affect the number of organisms, such as

temperature, aeration, and materials susceptible to nitrification. The number of organisms in two soils must be equalized before their capacity to nitrify can be compared.

It is important, in nitrification experiments, to know whether the difference in nitrification between two soils is due to differences in initial number of bacteria, to need or abundance of calcium carbonate, to a combination of both, or to other factors. It may then be necessary to attempt to equalize the differences. In certain types of experiments, soils of high nitrifying power, as determined by previous work, should be selected.

SUMMARY

A large number of samples of soils did not nitrify ammonium sulfate though they usually produced some nitrates from the soil nitrogen. Soils which do not nitrify ammonium sulfate may be caused to nitrify it by addition of cultures of actively nitrifying soil, of calcium carbonate, or of both nitrifying culture and calcium carbonate.

One-third of 21 surface soils have a high nitrifying power and one-third have practically no nitrifying power for ammonium sulfate by the method used. One-sixth of 32 subsoils had a high nitrifying power and 60 per cent had no nitrifying power for ammonium sulfate.

Approximately one-half of the 7 surface soils which did not nitrify ammonium sulfate could be made to have a high nitrifying power by additions of calcium carbonate, and the other half by additions of both calcium carbonate and soil cultures containing actively nitrifying bacteria. Of the 19 subsoils which did not nitrify ammonium sulfate, 2 assumed a high nitrifying power by additions of bacteria alone, 1 by addition of carbonate, and 16 by addition of both calcium carbonate and bacteria in a nitrifying culture.

Additions of sterilized cultures low in calcium carbonate did not bring about nitrification. The chief effect of the addition was due to the bacteria and not to other constituents of the added soil. The cultures were portions of soils receiving ammonium sulfate and nitrifying it to a high extent in a previous experiment.

Additions of inoculating liquid produced little nitrification. This is not a satisfactory method of adding nitrifying organisms to soils.

Nitrifying organisms may remain in a dry soil for many years.

The quantity of nitrates produced increased in general as the quantity of nitrifying cultures added to the soil increased from 0.1 to 20 gm. added to 200 gm. sterilized soil, but was not in direct proportion to the number of bacteria added. The quantity of nitrates is not suitable for use as a direct measure of the number of bacteria added.

The same quantities of nitrifying bacteria added to different soils in some cases produced different quantities of nitrates; this may be due to differences in the chemical composition of the soils.

The same sterilized soil inoculated with cultures of different soils in some

cases produced different quantities of nitrates; this may be due to differences in the nature or activity of the organisms in the different cultures.

Soils of high nitrifying capacity after sterilization sometimes produce more nitrates, with the same quantity of inoculant, than soils of low nitrifying capacity.

The nitrite organisms may be present in sufficient number to produce appreciable amounts of nitrites from ammonium sulfate when the nitrate organisms are not sufficiently abundant or active to produce nitrates.

Nitrites may be produced from ammonium sulfate when calcium carbonate is added, though few nitrates may be produced at the same time.

The quantity of nitrites from ammonium sulfate in a sterilized soil was found to be highest when 0.1 gm. of inoculant was used to 200 gm. of sterilized soil and decreased with the amount of inoculant, until, with 2.0 gm. of inoculant, practically no nitrite remained.

Nitrite organisms are either more abundant in the soil than nitrate organisms or else they multiply more rapidly.

The total production of nitrites, as measured by the sum of the nitrous and nitric nitrogen, is not in direct proportion to the numbers of organisms added. The first addition of 0.1 gm. of culture to 200 gm. of soil produces a high quantity of nitrites plus nitrates, whereas subsequent increments produce much smaller quantities.

When field soils are compared in nitrifying power for ammonium sulfate, the differences observed may be due chiefly to differences in the numbers of nitrifying bacteria at the time the experiment was begun. Abundance or deficiency in calcium carbonate may be another important factor.

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THE MICROFLORA OF THE ASH OF KATMAI VOLCANO WITH ESPECIAL REFERENCE TO NITROGEN FIXING BACTERIA

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The revegetation of volcanic areas or more precisely the colonization of volcanic ash which is inorganic and practically free from combined nitrogen offers opportunity for the study of many problems connected with the genesis of a soil not to be duplicated elsewhere. It was primarily with the hope of contributing to a knowledge of humus formation and of the "nitrogen cycle" that the National Geographic Society dispatched expeditions under the leadership of R. F. Griggs to the area devastated by the eruption of Katmai in 1912. But during the period covered by the five earlier expeditions, 1915 to 1919 inclusive (5, 7), the ash remained bare and nothing could be done with the main problem beyond laying the foundations necessary for understanding colonization when it should occur. The expeditions of these years, however, resulted in the discovery of the Valley of Ten Thousand Smokes and the publication of important contributions to an understanding of mineralization and volcanism.

After an interval of 11 years Griggs returned to Katmai, in 1930, again under the auspices of the National Geographic Society. This time the ash was found to be covered with a carpet of liverworts (*Jungermanniaceae*) in pure stand, as detailed elsewhere (6). Since the nitrogen content of the ash is very low, the possibility of nitrogen fixation at once suggested itself. This paper is an attempt to deal with that problem as far as it concerns nitrogen fixing microörganisms with which the liverworts might be associated.

METHODS AND MATERIALS

Previous to the departure of the expedition in 1930, the collection of samples of ash was discussed and the decision was made to bring back samples taken aseptically which could be analyzed bacteriologically and the flora compared in a general way with that of soil. All materials taken on the expedition or brought back had to be limited because of the fact that everything had to be transported several miles on mens' backs. Therefore, instead of having large samples, we had to be content with only a few grams of materials. The con-

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tainers were small test tubes which were plugged with cotton and sterilized before leaving Washington, D. C. In obtaining the samples, a test tube with the plug removed was pushed horizontally into the perpendicular cut surface of the ash. The test tube was then sealed off in an alcohol flame. As a control as to whether sealing the tube for 2 or 3 months would have any great detrimental effect on the microflora, garden soil from near Washington was sealed in this manner and carried to Katmai and return. Other samples of ash were taken in sterile tubes and replugged. These samples, of course, soon dried out and remained air dry until plated out several months later.

When the samples were received in the laboratory in Washington, D. C., 2-gm. portions were aseptically weighed out, dilutions made in sterile tap water in steps of 10, starting with a 1 to 50 dilution. From each of these dilutions five plates were poured with each of the various media employed.

The medium used for determining the number of microörganisms present in the ash was soil extract agar. This medium is the best one available, as it allows the greatest number of microörganisms to develop when compared to other substrates (21). It should not be considered perfect, for it is known that groups of bacteria, especially the autotrophic bacteria, will develop only on special media, making a universal medium out of the question.

The soil extract is prepared by autoclaving 500 gm. of air-dry field soil and 1,500 cc. of tap water for one-half hour at 15 pounds pressure. After the soil extract has been filtered clear through paper, 0.05 per cent K_2HPO_4 and 1 per cent agar are added, and the reaction is adjusted to pH 6.8, if necessary. The plates were incubated at 28°C. for 14 days.

For estimating the number of fungi in the ash samples, plates were poured with acid dextrose nitrate agar. This is prepared by adding to soil extract 0.1 per cent KH_2PO_4, 0.1 per cent $NaNO_3$, 2.5 per cent agar, and 1 per cent dextrose. The dextrose is added after the agar is melted and filtered through cheesecloth to remove any large particles of dirt. The reaction is adjusted to pH 4.2, by adding approximately 1 cc. each of normal HCl and H_2SO_4. Sterilization of this acid medium is accomplished by autoclaving 12 minutes at 12 pounds with the containers set in a water bath. This keeps the agar from breaking down too much and has been entirely safe as far as contaminations are concerned. The plates were incubated for 3 days at 28°C.

The medium used in pouring plates for actinomycetes consisted of soil extract to which was added 0.1 per cent K_2HPO_4, 0.1 per cent $NaNO_3$, 1 per cent glycerine, and 1 per cent agar. The reaction was adjusted to pH 6.8. Plates were incubated 10 days at 28°C.

Plates were also poured with Ashby's nitrogen-free agar (1) in order to see whether nitrogen-fixing bacteria were present in the ash. The ingredients of the medium were not purified by recrystallization, consequently traces of nitrogen were present, as is usually the case. These plates were incubated for 10 days at 28°C.

TABLE 1

Plate counts of groups of microörganisms in various samples of volcanic ash and the relative occurrence of B. radiobacter *among cultures isolated from Ashby's agar plates*

Per moist gram unless otherwise noted

NUMBER	DESCRIPTION	PLATE COUNTS	FUNGI	ACTINOMYCETES	MUCOID COLONIES	MUCOID COLONIES ISOLATED	IDENTIFIED AS *B. radiobacter*
1	Undisturbed ash, bare, 23	140,000	0	0	4,000	11	0
2	Fine ground ash, windblown and bare, 30	160,000	few	0	4,000	..	..
3	Windblown ash around roots of Calamagrostis, 22	70,000	0	0	20,000	10	0
4	Bright green liverwort layer, air dry, 4	65,000	50	0	3,250	8	8
5	Bright brown liverwort layer, air dry, 9	277,000	200	0	55,000	7	6
6	Black liverwort layer, sample kept in dark, butyric odor, 13	2,700	50	0	200	..	..
7	Black liverwort layer butyric odor, 15	1,000*	1,600	0	100*	..	..
8	1 inch below black liverwort layer; black coloration; butyric odor, 20	500,000	0	0	1,200	9	3
9	Duplicate of above, except no color and no odor, 20	900,000	450	0	1,000	..	..
10	6 inches below black liverwort layer, 21	1,400,000	0	0	60,000	9	2
11	Top ash with dead rhizoids, 29	300,000	0	0	8,000	10	8
12	2 inches below liverwort layer at Kodiak, 31	80,000	few	0	40,000	7	3
13	Lupine root and ash, 24	570,000	120	0	400,000	8	0
14	Ash near lupine roots, 25	1,600,000	40,000	0	150,000	9	2
15	Old soil from exposed crest, 28	65,000	50	0	11,000	4	0
16	Dry liverwort in envelope, not taken aseptically	72,000	31,000	0	2,750	7	7
17	Sealed soil, carried to Katmai and return, 19	9,200,000	8,000	1,700,000	6,000	..	..
18	Fresh soil from same locality	19,200,000	49,000	2,750,000	6,000	..	..

* Plates covered by fungi, accurate counts impossible.

NUMBERS OF MICROÖRGANISMS IN THE ASH

The plate counts on soil extract agar are given in the first column of table 1. The count of 140,000 microörganisms per moist gram of the undisturbed ash is very low if compared to a fertile soil similarly sealed and analyzed (number 17). Even lower counts than this were obtained in most of the samples of liverwort.

Of the five samples of liverwort plated out, two were taken in sterile test tubes and not sealed, the cotton plug being replaced in the tube, two were sealed and remained moist, and one was a sample brought in a package without precautions to avoid contamination by microörganisms. The numbers in the dry liverwort taken aseptically (numbers 4 and 5) were 65,000 and 277,000 whereas in the moist sealed samples (numbers 6 and 7) they were 2,700 and 1,000 or more. Since these moist sealed samples had a butyric odor it is logical to suppose that they had undergone some changes detrimental to the aerobic forms. The numbers in bulk sample number 16 agree well with those in sample 4, indicating that no contamination had occurred in the former while being transported in the paper envelope. Its dry condition would militate against any change in the flora.

Underneath the liverwort layer, larger numbers were found than were found in the liverwort layer. The sample taken 1 inch below (number 8) showed a black discoloration and had a butyric odor whereas the duplicate, number 9, showed no darkening and had no odor. The count of 900,000 in the latter is probably nearer the truth. In the sample taken 6 inches below the surface (number 10) still larger numbers were found. However, this count (1,400,000) is not as large as found in ordinary soil. The ash around lupine roots (number 14) contained about the same number of microörganisms, although the root with adhering ash contained only 570,000.

The soil, sealed and transported to Alaska and return (number 17), seems to have changed somewhat when compared with fresh soil taken from the same locality. The fresh sample, however, was taken after the return from the expedition and is hardly comparable to the sealed sample taken some months previously. Even if they were comparable, the reduction from 19,000,000 to 9,200,000 would not be very serious. It is quite possible that more change might have occurred in the sealed liverwort due to the relatively high percentage of organic matter.

THE NUMBERS OF FUNGI

The absence of fungi in the ash is very striking. Where liverwort is growing fungi seem to be able to exist. In number 7, the fungi overgrew all plates so that it was impossible to make anything like accurate counts of the mucoid colonies or of the total numbers. The dry bulk sample (number 16) showed by far the highest number of fungi among the liverwort samples (31,000), the other dry samples (numbers 4 and 5) containing only 50 and 200 respectively. If this were not due to chance sampling it may be that the large bulk dried out more slowly than the small samples in the test tubes thereby giving the fungi a chance to form spores. The ash near the lupine roots contained 40,000 fungi per gram whereas the lupine root with adhering ash contained only 120.

As stated in the foregoing, numbers 17 and 18 are not comparable, inasmuch as the fresh soil sample (number 18) was taken several months later than number 17. However, it is probable that some reduction in numbers took

place due to the sealing of the soil sample (number 17). But it is not probable that the absence of fungi from the ash and the low numbers in the liverwort can be due to this cause.

THE ABSENCE OF ACTINOMYCES IN THE ASH

No actinomycetes were found in any of the ash or liverwort samples. This probably means that conditions were not favorable for their development. It is not conceivable that inoculation of the ash had not occurred during the 18 years' exposure to the elements.

Although the actinomycete group has been studied considerably in the laboratory (23) very little is known of their physiology under natural conditions. In ordinary soil they comprise from 15 to 40 per cent of the total count depending upon the crop grown and soil treatment. The highest numbers are found under sod (23, p. 40). One would expect to find some in sample number 15, old soil from exposed crest. Their absence is inexplicable.

BACTERIA DEVELOPING ON NITROGEN-FREE MEDIA

The azotobacter group produce large, easily recognized colonies on Ashby's agar, whereas the colonies of the root-nodule bacteria (*Rhizobia*) are smaller and not distinguishable from colonies of other bacteria. Both the *Rhizobia* and these other bacteria produce more or less mucoid colonies on Ashby's agar. Their growth means that they are either using the nitrogen of the air or can grow with the very small amounts of nitrogen present in the medium as contamination. Undoubtedly many bacteria not belonging to the azotobacter group fix a little nitrogen when conditions are right. On the other hand, it should not be taken for granted that if colonies grow on Ashby's medium they fix nitrogen.

No colony of *Azotobacter* was seen on any of the plates made from the ash or liverwort. But since some bacteria appeared to grow on the nitrogen-free medium it was thought advisable to make isolations from the plates and to observe the cultures more closely. Accordingly several of the most vigorous growing colonies were picked from the Ashby agar plates, and inoculated into the same medium. After 2 days' growth, smears were stained by the Gram method. If the cultures were pure, transplants were made to beef extract agar, milk, gelatin, and glycerine nitrate agar.

The isolations made from the undisturbed ash (number 1, table 1) were all gram-negative small rods, growing weakly on Ashby's agar and as a flat gray growth on beef extract agar, coagulating and sometimes digesting milk. Four of the eleven liquefied gelatin. From these observations, it was concluded that nothing could be gained by further study of these cultures since none of them resembled any of the ordinary nitrogen-fixing bacteria. Similar bacteria were isolated from the windblown ash (number 3).

The observations on the eight mucoid cultures isolated from the bright green liverwort layer (number 4) showed that all of the cultures belonged to the *B.*

radiobacter group. Similar results were obtained from plating the sample of the bright brown layer of liverwort (number 5), the bulk sample of liverwort (number 16), and the top ash with dead rhizoids (number 11).

No isolations were made from the moist sealed liverwort samples (numbers 6 and 7) because the plates were overgrown by fungi.

It is also interesting to note that from the sample taken 1 inch below the liverwort (number 8), three of the nine isolations were *B. radiobacter*; from 2 inches below (number 12), three of the seven; and from 6 inches below (number 10), two of the nine.

It is rather surprising that no colonies of *B. radiobacter* were included in the eight mucoid colonies which were picked from the plates made from lupine roots (number (13). This organism is abundant around the roots of legumes growing in soil (20). In this case, it is possible that *B. radiobacter* was outnumbered by other bacteria on account of the decay of the roots and did not appear in such high dilutions. The fact that two of the nine colonies isolated from ash near the roots (number 14) proved to be *B. radiobacter* seems to support this assumption.

No colonies of *B. radiobacter* were found on the plates made from the old soil of exposed crest (number 15) although the number of colonies on Ashby's agar was noticeably high. The four cultures isolated were gram-positive spore formers.

Cultures of liverwort grown by one of us (R. F. G.) on nitrogen free agar or in solution always have *B. radiobacter* as a contamination. The growth of the bacterium is scant and often not discernible in solutions. Whether its presence on the rhizoids is significant can not be stated at this time.

B. radiobacter is a soil bacterium closely related to the nodule-bacteria (*Rhizobia*), to the crown gall organism (*Phytomonas tumefaciens*), and to *Aerobacter aerogenes*. It has been found to be more numerous under sod than in bare soil (16), and is generally more numerous in soil on which legumes are growing (20), although certain non-legumes, as rape (22), seem to increase the number also.

The function of *B. radiobacter* in the soil has not been definitely determined. In pure cultures in the laboratory, small amounts of nitrogen have been fixed in a number of cases (3, 4, 9, 10. 11, 13, 14, 19) whereas in other cases (2, 18, 19) nitrogen was not fixed or was fixed by some cultures and not by others. Some have even reported a loss of nitrogen from the culture solution, especially if some nitrate is present (2, 3, 4, 12, 18). This has led to the conclusion that *B. radiobacter* has three distinct physiological reactions, viz., nitrogen fixing, nitrate assimilating, and nitrate reduction.

NITROGEN FIXATION EXPERIMENTS

Two flasks containing 50 cc. of Ashby's solution and two flasks of Ashby's solution with 1 per cent dextrose substituted for mannite were inoculated with about half a gram of sample numbers 1, 3, 6, 9, 10, 11, 14, 15, 17 and with

a control fresh soil sample. No visible growth occurred in either solution when inoculated with the samples of ash or liverworts. Sample number 17 and the control soil gave a good growth and each had an azotobacter film. The nitrogen fixed in Ashby's solution by number 17, the sealed soil sample, and by the fresh control soil was 4.7 and 3.4 mgm., respectively; when dextrose was substituted for mannite in that solution it was 3.8 and 2.6 mgm., respectively. The flasks inoculated with number 6, the black liverwort layer, and those inoculated with number 11, ash with dead rhizoids were also analyzed for total nitrogen. As no growth took place in these flasks, no fixation was anticipated and none was found.

In view of the fact that *B. radiobacter* has been reported as fixing nitrogen, it was thought advisable to try pure cultures isolated from a liverwort sample. Since this organism apparently did not grow in Ashby's solution, as has been noted, soil extract with 0.05 per cent K_2HPO_4 and 1 per cent mannite was used for this test. The amount of growth which developed in 15 days was not very great, although there was a definite development of the organism. Analysis for total nitrogen gave 0.1, 0.17, and 0.15 mgm., above the control per 100 cc. solution. This apparent fixation probably can be explained by the inaccuracies of the modified Kjeldahl method in such work (8) or can be attributed to a loss of nitrogen from the control medium (15) or to some other cause.

Of course, the possibility should not be overlooked that under different cultural conditions nitrogen might be fixed by pure cultures or that nitrogen might be fixed by the liverwort and *B. radiobacter* in symbiosis.

The nitrogen fixing microörganisms occurring in disintegrating lava on the slopes of Vesuvius were studied by Riccardo (17). Pure cultures of a Mycoderma showed a fixation of nitrogen ranging from 1.8 mgm. to 3.4 mgm. per 100 cc. solution in five of nine flasks, the other four flasks failing to show any increase in nitrogen. Only one of the three flasks inoculated with an organism identified as *B. amylobacter* showed any nitrogen fixation, whereas seven of the eight flasks inoculated with a bacterium which he believed to belong to the genus Azotobacter showed a gain of nitrogen ranging from 1.1 mgm. to 2.6 mgm. per 100 cc. of solution.

Although the lavas studied by Riccardo were still devoid of higher plants, they were considerably disintegrated from exposure to the weather for about 250 years. Inasmuch as the ash of Katmai was only 18 years old when sampled, it is obvious that the conditions obtaining there are not comparable with those in the weathered lava.

SUMMARY

Samples of bare volcanic ash collected 18 years after the eruption of Mt. Katmai contained no fungi or actinomycetes. The number of bacteria was about 150,000 per gram and comprised none of the organisms commonly known to be able to fix nitrogen.

Samples of ash on which a layer of various species of liverworts (*Jungerman-*

niaceae) were growing were plated out with the following results: Fungi were usually present in small numbers; no actinomycetes were found; the numbers of bacteria found in the surface layer varied from 65,000 to 300,000, 1 inch below the layer 900,000, and 6 inches below, 1,400,000.

B. radiobacter was readily isolated from Ashby agar plates made from the liverwort layer. It was also found underneath the liverwort but to a less degree. No culture of *Azotobacter* was obtained nor was there any development of azotobacter slime in Ashby's solution.

Nitrogen fixing experiments using Ashby's solution and inoculated with the ash or liverwort layer, failed to show any fixation, whereas a fresh soil and a sample sealed like the ash samples both gave a fair fixation and the typical azotobacter pelicle.

Pure cultures of *B. radiobacter* grown in a soil extract mannite solution also failed to fix appreciable amounts of nitrogen.

Therefore it is concluded that if fixation of nitrogen occurs in volcanic ash, it can not be attributed to the microflora.

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NITROGEN-FIXERS OF LEACHED ALKALI SOILS[1]

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Most Utah soils studied, if incubated with optimum moisture and an appropriate carbohydrate, fix nitrogen (1). Many of these soils on incubation, without the addition of a carbohydrate, gain in nitrogen. A few of the soils studied, if mixed with plant residues and kept in a greenhouse, fix appreciable quantities of nitrogen (4). The quantity of nitrogen gained varies with the specific soils, the plant residues added, and the specific salts within the soil. The microörganisms which take part in the nitrogen fixation are quite resistant to so-called "alkali salts" (2), and some "alkali salts" accelerate the rate at which the soils gain nitrogen. A soil may gain considerable nitrogen under these specified conditions and still not give a typical Azotobacter membrane when inoculated into Ashby media (3). This paper considers some comparatively active nitrogen-fixing microörganisms which have been obtained from three "alkali soils:" (*a*) the college farm soil which had been rendered unproductive by the addition of 0.66 per cent each of sodium chloride, sodium sulfate, and sodium carbonate; (*b*) a Corinne soil which, before being leached, was so high in soluble salts, principally chlorides, that it was barren; and (*c*) a Richland Acres soil which contained sufficient soluble salts, principally sulfates, to prevent the growth of most plants. Each of these soils had been leached with water until the major portion of the soluble salts was removed before the microörganisms were isolated. After being leached, the soils had produced small crops of crimson clover and barley (6).

The pure cultures were obtained from Ashby agar by repeated dilutions, plating, and microscopic examinations. Their nitrogen-fixing ability was determined by incubation in sterile soil to which an appropriate carbohydrate had been added. The stock cultures were kept on the soil extract, Ashby agar. This soil extract was prepared by extracting 2 gm. of soil with 100 cc. of distilled water. The microörganisms were studied both morphologically and physiologically according to the descriptive chart prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists

[1] Contribution from department of bacteriology and chemistry, Utah Agricultural Experiment Station.

[2] Bacteriologist and graduate student, respectively.

The authors wish to express their appreciation to Milton Nelson for assistance in the nitrogen determinations.

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(9). A careful study of the literature and related organisms makes it highly probable that these are new species or at least new varieties. However, we are merely designating the organisms by a number and giving the main morphological and physiological characteristcs.

Bacterium 16b. Gram-positive, non-spore-forming rods 0.6 to 0.7 by 1 to 1.7 μ, occurring singly.

Gelatin colonies: Rapid, punctiform, cream, entire, coarsely granular growth.

Gelatin stab: Liquefaction crateriform, filiform with good surface growth, medium unchanged.

Agar colonies: Rapid, circular, smooth, raised, entire, finely granular.

Agar slant: Abundant, light yellow, glistening, raised, contoured, butyrous growth.

Broth: Slight clouding, no sediment.

Litmus milk: Neutral with slow peptonization. Litmus reduced.

Potato: Abundant, yellow-tan growth, potato darkened.

Produces indole, reduces nitrates with the formation of gas. Produces amonia from peptone, hydrolyzes starch, produces acid on glucose and sucrose but not on lactose. Aerobic. Habitat, College Farm soil.

Micrococcus 17b. Gram-negative, non-motile cocci 0.8 to 1 μ, occurring in sheets and in clusters.

Gelatin colonies: Slow, punctiform, raised, entire, finely granular, cream-colored growth.

Gelatin stab: Villous, no liquefaction.

Agar colonies: Rapid, circular, punctiform, smooth, raised, entire, finely granular light pink growth.

Agar slant: Abundant, echinulate, raised, glistening, contoured, opaque, butyrous growth.

Broth: No surface growth, slight clouding with scanty flocculent sediment.

Litmus milk: No change.

Potato: No growth.

Reduces nitrates and produces acid on lactose and glucose but not on sucrose. Produces ammonia in peptone. Aerobic. Habitat, College Farm soil.

Bacillus 6b. Gram-positive rods 0.9 to 1.2 by 1.9 to 3.3 μ, occurring in chains. Motile by means of monotrichous flagellum. Spores terminal, spherical 0.5 to 0.7 μ. Gram-positive

Gelatin stab: Liquefaction crateriform.

Agar colonies: Rapid, circular, smooth, raised, entire, finely granular, pink colonies.

Agar slant: Abundant, peach-colored, glistening, opaque, contoured, butyrous growth.

Broth: No clouding, no surface growth.

Litmus milk: Slightly acid.

Potato: No growth.

Hydrolyzes starch. Produces indole. Reduces nitrates but does not produce acid on glucose, lactose, or sucrose. Produces ammonia in peptone solution. Aerobic. Habitat, College Farm soil.

Bacterium 119b. Gram-positive, non-motile, non-spore-forming rods 0.5 to 0.7 by 1.5 to 1.9 μ occurring in clusters.

Agar slant: Abundant, echinulate raised, glistening, smooth, opaque, butyrous growth.

Gelatin stab: Liquefaction crateriform.

Broth: No clouding or surface growth.

Does not produce indole nor reduce nitrates. Produces ammonia in peptone. Hydrolyzes starch, produces acid on glucose, but not on lactose or sucrose. Aerobic. Habitat, Corinne alkali soil.

Micrococcus 115a. Gram-positive, non-spore-forming cocci 0.5 to 0.8 μ, occurring in pairs and clusters.

Gelatin colonies: Slow, punctiform, raised amorphous with entire edges.

Gelatin stab: Liquefaction crateriform.

Agar colonies: Rapid, circular, smooth, raised, amorphous with entire edges.

Agar slant: Abundant, echinulate, convex, glistening, smooth, opaque, butyrous growth.

Broth: Slight clouding.

Litmus milk: Alkaline slow peptonization.

Potato: Abundant, tan, spreading growth.

Produces indole, does not reduce nitrates but produces ammonia from peptone and urea. Hydrolyzes starch but does not produce acid from dextrose, sucrose, or lactose. Aerobic. Habitat, Corinne alkali soil.

Archromobacter 11a. Gram-negative, non-motile rods 0.5 to 0.6 by 2.4 to 6 μ, occurring singly and in clusters.

Gelatin colonies: Slow, small, punctiform, raised, entire finely granular with cup-like liquefaction.

Gelatin stab: Slight liquefaction. Good surface growth. Medium unchanged.

Agar colonies: Rapid, punctiform, smooth, raised, entire, coarsely granular colonies.

Agar slant: Milky, abundant, echinulate, glistening, contoured, opaque, butyrous growth.

Broth: No clouding, no pellicle formation.

Litmus milk: Alkaline with slow peptonization.

Potato: Slight, raised, milky growth.

Produces indole, does not reduce nitrates nor ammonify peptone. Aerobic. Habitat, College Farm soil.

Micrococcus 114d. Gram-positive, non-motile, non-spore-forming cocci 1.2 to 1.6 μ, occurring in pairs and clusters.

Gelatin colonies: Slow, irregular, raised, coarsely granular with lobate edges and saucer liquefaction.

Gelatin stab: Liquefaction saccate. Reddish brown in color.

Agar colonies: Rapid, punctiform, smooth, raised, finely granular with entire edges.

Agar slant: Moderate, raised, glistening, opaque, cream-colored growth.

Broth: Slight clouding and very scanty sediment.

Does not produce indole, reduce nitrates nor hydrolyze starch. Produces ammonia in peptone. Aerobic. Habitat, Corinne alkali soil.

Bacterium 202b. Gram-negative, non-motile, non-spore-forming rods 0.5 to 0.7 by 1.2 to 1.9 μ. occurring singly and in clusters.

Gelatin colonies: Slow, punctiform, cream-colored.

Gelatin stab: Liquefaction napiform.

Agar colonies: Rapid, circular, smooth, raised with entire edges.

Agar slant: Abundant, glistening, raised, opaque growth.

Broth: Moderate clouding with flocculent surface growth and sediment.

Litmus milk: Alkaline with slow peptonization, litmus reduced.

Potato: Abundant tan growth. Medium darkened.

Produces indole. Does not reduce nitrates. Hydrolyzes starch. Produces acid in dextrose media. Aerobic. Habitat, Richland Acres alkali soil.

Actinomyces 227a. Mycelium straight with little branching.

Gelatin colonies: Rapid, raised filamentous.

Gelatin stab: Liquefaction crateriform.

Agar colonies: Rapid, irregular, raised undulate, radially ridged, coarsely granular.

Agar slant: Abundant, echinulate, dull, raised rugose, translucent brown, butyrous growth.

Broth: Slightly cloudy, with abundant flocculent sediment and surface growth.

Litmus milk: Acid with peptonization.

Potato: Moderate white growth. Potato darkened around growth.

Produces indole. Reduces nitrates. Produces acid on glucose. Hydrolyzes starch and actively decomposes cellulose. Aerobic. Habitat, Richland Acres alkali soil.

Bacterium 16e. Gram-positive, non-motile, non-spore-forming rods 0.5 to 0.6 by 0.7 to 0.9 μ, occurring singly and in clusters.

Gelatin stab: Liquefaction crateriform.

Agar colonies: Rapid, circular, smooth, flat, entire, ivory-colored, finely granular.

Agar slant: Abundant, echinulate, raised, glistening, ivory-colored growth.

Broth: Moderate clouding, no pellicle formation.

Litmus milk: Slightly acid with slow peptonization.

Potato: Light creamish tan, abundant growth. Potato darkened.

Produces indole. Slowly ammonifies peptone but does not reduce nitrates nor hydrolyze starch. Produces acid on glucose but not on sucrose or lactose. Aerobic. Habitat, College Farm soil.

Micrococcus 4. Spheres 0.7 to 1.2 μ, occurring in clusters and pairs. Gram-negative.

Gelatin colonies: Very small, punctiform, slow to develop, raised, entire, finely granular colonies.

Gelatin stab: Liquefaction crateriform slow surface growth.

Agar colonies: Rapid, circular, smooth, raised, entire, amorphous.

Agar slant: Abundant echinulate, raised, glistening, opaque growth.

Broth: Slightly clouded, no surface growth.

Litmus milk: Alkaline with slow peptonization.

Potato: Abundant light tan growth.

Does not produce indole nor reduce nitrates. Hydrolyzes starch but does not produce acid on glucose, sucrose, or lactose. Produces ammonia in peptone solution. Aerobic. Habitat, College Farm soil.

The nitrogen-fixing powers of the various organisms were determined as follows: One-hundred-gram portions of soil pulverized and passed through a sieve with round holes 1 mm. in diameter were weighed into 500-cc. Erlenmeyer flasks, the moisture made up to 20 per cent and stoppered with cotton. They were then autoclaved for $2\frac{1}{2}$ hours at 120°C., after which 10 cc. of sterile distilled water containing 1.0 gm. of the specific carbohydrate was added to each flask. Bacteriological tests were made on the soils to make certain they were sterile, after which they were inoculated in triplicates with the specific organism to be tested. Sterile checks were run in both these and all other series reported. All were incubated for 5 weeks at 28°C., the moisture content being maintained with sterile distilled water at approximately 26 per cent. At the end of 5 weeks the samples were dried at 120°C., pulverized, sieved as before, and the total nitrogen was determined by Official Gunning method (8, p. 8). Blanks were run on all chemicals and a high grade of sodium hydroxide was used, thus preventing frothing and loss of nitrogen. The results, as reported in table 1, are the average of six or more closely agreeing determinations. They represent the nitrogen gains in milligrams for 10 gm. of soil. The errors due to analyses have been calculated according to the formula, P.E. $= .67\sqrt{\frac{\Sigma d^2}{n^2}}$; where the value is $\pm$ .04 or less it is reported as $\pm$0.0, where $\pm$ .05 to $\pm$ 0.1 it is reported as $\pm$ 0.1

The 11 organisms fixed appreciable quantities of nitrogen, the quantity varying with the specific organism and the carbohydrate at its disposal. The average fixation was highest on sucrose and arabinose. Usually only small

quantities of nitrogen were fixed where xylose, maltose, or dextrose furnished the energy. Starch and inulin are quite acceptable sources of energy to the majority of the microörganisms. Mannite and sodium lactate are only fair sources of energy. It is interesting to note that Löhnis and Pillai (7) found dextrose to be only one-half as valuable to Azotobacter as is sucrose. They found starch low in value to Azotobacter, whereas in the experiment reported it is quite acceptable. These organisms appear to be from one-third to two-thirds as efficient in the fixation of nitrogen on the various carbohydrates as Löhnis and Pillai found the Azotobacter.

The soil in which these organisms have been cultured contains, as determined by the official Kjeldahl method, 0.132 per cent nitrogen; when determined by the absolute, or cupric oxide method (8, p. 9), however, it yielded 0.159 per cent nitrogen, which shows that only 83 per cent of the nitrogen carried by this soil is obtained by the Kjeldahl method. Therefore, each 100 gm. of soil contains 27 mgm. of nitrogen which is not determined by the latter method. Consequently, it may be argued that the gains in nitrogen obtained when this soil is sterilized, inoculated with the specific microörganism, and then incubated, result from the transforming of the non-determinable pyrrole nitrogen and the small quantity of nitrates present into nitrogen compounds of such a nature that it can be determined by the Kjeldahl method. If this were true, the gains in nitrogen would be only apparent and the organisms would be transforming nitrogen compounds but would not be fixing atmospheric nitrogen. In order to test this supposition 10 of the organisms were inoculated separately into sterile sucrose-containing soil. These were incubated for 5 weeks at 28–30°C. with optimum moisture content. Along with these were incubated sterile sucrose-containing soil. At the end of the incubation period the soils were dried and the nitrogen determined by the absolute method.

The average fixation in this set (table 2) was slightly higher than in the former. The order of efficiency changes, but in every case except that of *Micrococcus* 17b there were appreciable gains of nitrogen. Nor could it be argued that this nitrogen came from combined nitrogen, ammonia, nitrous and nitric acid, taken from the air since beside each inoculated flask was a similar sterile flask which was used for a check.

The observed gains in nitrogen are small yet significant, as is evident from the following facts: (*a*) Extreme care has been taken in the nitrogen determinations. Blank checks were run on all chemicals and determinations made on sterile soil containing the same quantity of water and carbohydrate and incubated alongside of the inoculated soil; the reported gains are usually considerably more than three times the experimental error. (*b*) More than 200 uniformly positive results have been obtained on these organisms when grown on various carbohydrates. (*c*) When the soils from which these organisms were obtained were kept under carefully controlled conditions in a greenhouse with optimum moisture and an appropriate source of energy, carbohydrate, or plant residue, for from 3 to 5 years, they gained appreciable quantities of

TABLE 1

Milligrams of nitrogen fixed in 10 gm. of soil containing various carbohydrates and inoculated with pure cultures of nitrogen fixers

	SUCROSE	ARABINOSE	INULIN	STARCH	LACTOSE	GALACTOSE	MANNITE	SODIUM LACTATE	XYLOSE	MALTOSE	DEXTROSE
Bacterium 16b	1.0±0.1	1.2±0.2	0.6±0.0	0.8±0.0	0.8±0.1	1.0±0.0	1.0±0.2	0.4±0.0	0.2±0.2	0.2±0.1	−0.2±0.2
Micrococcus 17b	0.8±0.2	0.6±0.0	0.4±0.1	0.2±0.1	0.4±0.2	0.4±0.0	0.4±0.2	0.8±0.0	0.6±0.0	0.4±0.0	0.4±0.0
Bacillus 6b	0.2±0.0	0.2±0.0	0.8±0.0	1.0±0.1	0.6±0.0	0.6±0.2	0.2±0.0	0.6±0.0	0.2±0.0	0.8±0.0	0.4±0.0
Bacterium 119b	0.6±0.0	0.6±0.0	0.6±0.1	0.8±0.1	0.6±0.0	0.8±0.2	0.6±0.0	1.2±0.1	0.2±0.1	0.0±0.1	0.0±0.0
Micrococcus 115a	1.2±0.2	0.8±0.1	0.4±0.1	0.4±0.0	0.6±0.1	0.4±0.1	0.4±0.1	0.6±0.2	0.6±0.2	0.2±0.1	0.4±0.1
Achromobacter 11a	0.6±0.2	0.6±0.1	0.6±0.1	1.0±0.2	0.8±0.0	0.8±0.1	0.2±0.2	0.6±0.1	0.6±0.1	0.4±0.2	0.4±0.1
Micrococcus 114d	1.0±0.0	1.2±0.2	0.8±0.0	0.6±0.3	0.6±0.0	0.2±0.1	0.2±0.1	0.6±0.0	0.0±0.1	0.0±0.2	0.4±0.1
Bacterium 202	1.0±0.1	0.8±0.0	0.8±0.1	0.6±0.1	0.6±0.2	0.4±0.0	0.6±0.2	0.2±0.2	0.2±0.2	0.0±0.1	0.0±0.1
Actinomyces 227a	0.6±0.1	1.0±0.1	0.6±0.0	0.4±0.0	0.6±0.1	0.4±0.0	1.0±0.0	0.0±0.1	0.0±0.1	0.0±0.1	0.2±0.1
Bacterium 16e	0.8±0.1	1.0±0.0	0.4±0.0	0.8±0.0	0.8±0.1	0.6±0.1	0.6±0.0	0.2±0.2	0.0±0.0	0.2±0.0	−0.4±0.1
Micrococcus 4	0.6±0.2	0.2±0.1	0.6±0.0	0.4±0.0	0.8±0.1	0.6±0.1	0.6±0.1	0.8±0.0	0.2±0.1	0.2±0.1	0.4±0.2

nitrogen. Yet some of these soils do not harbor known species of Azotobacter, nor have we been successful after long and careful search in finding known species of nitrogen-fixers in the soil. It is not uncommon to find soils devoid of Azotobacter which gain nitrogen just as rapidly as other soils containing Azotobacter. Therefore, we have reached the conclusion that the increase of nitrogen observed in these soils under field and greenhouse conditions is due to the cumulative action of numerous microörganisms of low nitrogen-fixing efficiency and not to a few extremely active species.

TABLE 2

Nitrogen fixed in 10 gm. of soil containing 1 per cent of sucrose during an incubation period of 5 weeks

Nitrogen determined by the absolute combustion method

MICROÖRGANISMS	NITROGEN FIXED IN 10 GM. SOIL
	mgm.
Bacillus 6b	1.4±0.2
Actinomyces 227a	1.4±0.1
Micrococcus 115a	1.2±0.1
Micrococcus 4	1.2±0.1
Bacterium 16b	1.0±0.0
Micrococcus 114d	1.0±0.1
Bacterium 16e	0.8±0.1
Achromobacter 11a	0.8±0.1
Bacterium 202	0.8±0.1
Micrococcus 17b	0.2±0.2

SUMMARY

Eleven microörganisms were obtained from leached alkali soils and studied in pure cultures; all of these possess the power of fixing nitrogen when cultured in soil with an optimum moisture content and appropriate carbohydrates. The quantity of nitrogen fixed varied with the specific microörganisms and the carbohydrate added to the soil. Sucrose, arabinose, inulin, starch, lactose, galactose, mannite, sodium lactate, xylose, maltose, and dextrose were all used by some of the organisms, the efficiency varying in the inverse order from which they are named. Xylose, maltose, and dextrose were poor sources of energy. Some of the organisms fixed as high as 1.4 mgm. of nitrogen in 10 gm. of soil. This compares favorably with fixations made by Azotobacter.

The possibility of the noted gains of nitrogen coming from pyrrole compounds or from combined nitrogen of the air are considered and found not to be the source of the gains.

The soils from which these microörganisms were obtained when kept in pots under greenhouse conditions with optimum moisture content gain appreciable quantities of nitrogen, most of which has been attributed to gains from atmospheric sources (4), and it is believed that the described organisms play a major rôle in the observed phenomenon.

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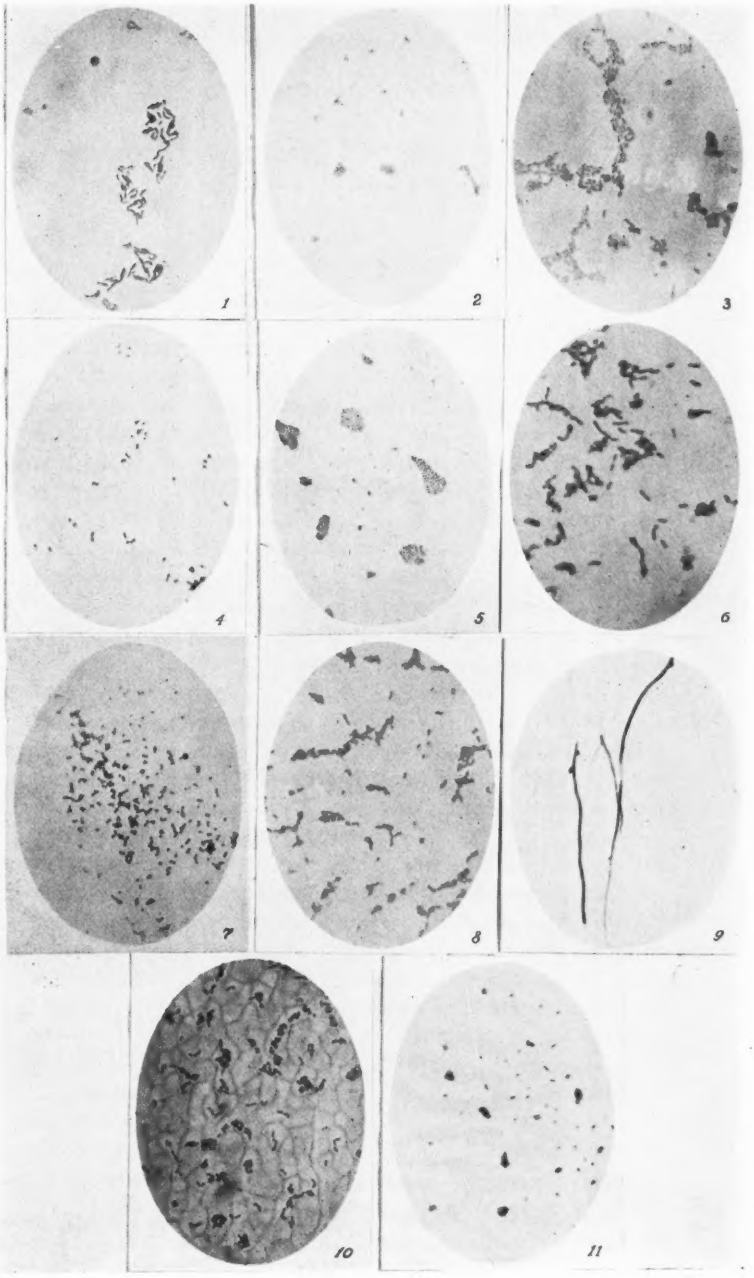
PLATE 1

MICROÖRGANISMS FROM LEACHED ALKALI SOIL

FIG. 1. *Bacterium* 16b.
FIG. 2. *Micrococcus* 17b.
FIG. 3. *Bacillus* 6b.
FIG. 4. *Bacterium* 119b.
FIG. 5. *Micrococcus* 115a.
FIG. 6. *Achromobacter* 11a.
FIG. 7. *Micrococcus* 114d.
FIG. 8. *Bacterium* 202.
FIG. 9. *Actinomyces* 227a.
FIG. 10. *Bacterium* 16e.
FIG. 11. *Micrococcus* 4.

All slides were made from 3-day-old cultures, grown on nutrient agar P.H. 7.4; stained with carbol fuchsin, magnification 1100×.



FIGS. 1–11

EFFECT OF EXCHANGEABLE BASE AND SOIL TREATMENTS ON PHOSPHORUS SOLUBILITY[1]

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From farm practice and experimental work it appears that the availability of phosphorus to plants, when applied as a fertilizer, varies greatly with the soil and possibly other factors. The literature contains many conflicting statements concerning phosphorus solubility and availability. The concensus of opinion seems to be that in acid soils the phosphorus is combined with iron and aluminum, and as a result of the insolubility of these phosphates the acid soils are likely to be high in total but low in soluble phosphorus. On the other hand, in neutral or basic soils the phosphorus will combine with calcium or magnesium and thus be more soluble and more available.

Comber (4) made the concise statement, "The removal of certain anions, such as phosphate ions from solution is a consequence of the exchange of bases between the soil and the solution." Double exchange may produce either a soluble or insoluble phosphate salt. Nemec and Gracinin (9) cited many conflicting statements on the resorption of phosphorus from soils, and concluded that increased adsorption of phosphorus under the influence of calcium carbonate had been demonstrated only on acid soils; and further that soils with adequate supplies of calcium show a depression in the resorption of phosphorus when treated with calcium carbonate.

Nemec (8) reported that soils lacking calcium and containing soluble iron do not respond to phosphorus fertilization even when they may be deficient in phosphorus. Soils with adequate calcium should have had more available phosphorus than soils lacking in calcium. Smirnov (11) found that saturating a degraded chernozem with Na, K, or NH_4 increases the amount of soluble phosphorus. Spurway (12), on treating a soil with KCl, $MgCl_2$, and $CaCl_2$ to exchange the bases, found that potassium treatments always, and the magnesium treatments generally, increase the solubility of the phosphorus, whereas the calcium treatments increase the solubility in acid soils and decrease it in neutral and alkaline soils.

Stephenson and Powers (13) found that applications of sulfur to soils increase the acidity and decrease the water-soluble phosphorus. Others have reported

[1] Contribution No. 169, department of chemistry.

[2] It is with pleasure that the cooperation of W. L. Latshaw in obtaining the data in table 1 is acknowledged.

that increased acidity causes decreased phosphorus solubility. Askinazi and Yarusov (2) showed by weak acid extractions, that liming increases the solubility of P_2O_5 in podzol soils. Demolon and Barbier (5) found that P_2O_5 is precipitated in an alkali soil lacking $CaCO_3$ but containing Ca, and in acid soils by means of iron sesquioxide. They also reported that liming acid soils without excess rather than acidifying increases the P_2O_5 mobility. Wityn (15) concluded that alkali materials increase the solubility of ferric and aluminum phosphates, and in acid soils liming increases the solubility of phosphates by changing them to calcium phosphate. Antonov (1) reported that applications of CaO increase the pH values and water-soluble phosphorus in certain soils.

Kappen and Bollenbeck (7) found that in acid soils of the same pH values, phosphorus solubility is greater when the acidity is due to neutral salt decomposition rather than to hydrolytic acidity.

No thorough review of the literature on this problem is necessary, as this has been done by others, notably Williams (14). The purpose of this research has been to investigate the rôle that exchangeable bases play in phosphorus solubility with and without the application of fertilizers and soil amendments.

SOILS USED AND TECHNIQUE EMPLOYED

The soil used in this work was Cherokee loam, obtained from Cherokee County, Kansas. This is a mineral soil which naturally responds to phosphate fertilization. Samples of the soil were prepared by replacing the exchangeable cations with Al^{+++}, Fe^{+++}, Ca^{++}, Mg^{++}, H^+, K^+, and NH_4^+. This was accomplished by successive leachings of the soil with normal solutions of the various chlorides except hydrogen where a twentieth normal solution was used. The soils were treated until the leachings contained no Ca^{++}. An untreated portion of soil was also retained for use. These soils will be referred to as aluminum, iron, calcium, magnesium hydrogen, potassium, ammonium, and untreated soils.

Gedroiz (6) states that exchangeable calcium is held more tenaciously than the other bases; so it is assumed that when the calcium is replaced, equilibrium is reached. Replacement in the soil treated with calcium chloride was judged to be complete when the magnesium chloride treated soil had reached equilibrium. After total replacement had been accomplished the soils were repeatedly washed with water until the soluble chlorides were removed or reduced to such a low concentration that sedimentation would not occur rapidly. By washing with water alone, the soils treated with iron and aluminum were completely freed from soluble chlorides. The soils that had been treated with potassium, ammonium, hydrogen, calcium, and magnesium chlorides, after being washed with water were then washed with 80 per cent alcohol. By the alcoholic washings the calcium, magnesium, hydrogen, and ammonium soils were entirely freed from soluble chlorides. The potassium soil was prac-

tically freed from soluble chlorides, for the last wash recovered before sedimentation failed to occur contained only a very few parts of chloride per million. The soils thus prepared were treated with various quantities of $CaH_4(PO_4)_2$, $CaCO_3$, $CaCl_2$, and KCl, applications being at a definite rate per acre on the basis of 2,000,000 pounds of soil per acre, which will be spoken of as fertilizer treatments. The composition of the soils treated with several bases is given in table 1.

The soluble phosphorus was determined by the coeruleomolybdate method of Deniges as adapted by Atkins (3). The phosphorus solution was obtained by shaking the soil with water and then centrifugalizing the suspension, which was then passed through clay filters, the phosphorus being determined in the filtrate. To overcome the retention of phosphorus by the clay filter, which Parker (10) and others found to occur, the soil particles were rinsed from the filter, and then 45 cc. of 0.05 *N* HCl was passed through the filter, the phosphate being determined in the mixture of filtrates. Before the filters were

TABLE 1

Composition of Cherokee loam treated with various bases

BASE SOIL WAS TREATED WITH	PERCENTAGE COMPOSITION							
	SiO_2	P_2O_5	Fe_2O_3	Al_2O_3	CaO	MgO	K_2O	NH_4
Fe	82.35	0.11	3.81	6.93	0.21	0.54	0.68	0.03
Al	82.20	0.11	2.62	8.12	0.23	0.54	0.68	0.03
Ca	82.42	0.11	2.63	7.52	0.58	0.56	0.70	0.03
Mg	82.20	0.11	2.93	?	0.20	0.99	0.70	0.03
NH_4	82.12	0.10	2.93	7.34	0.19	0.55	0.62	0.13
K	82.41	0.11	2.85	7.12	0.21	0.73	1.18	0.03
H	82.22	0.11	2.84	6.97	0.25	0.60	0.72	0.03
Untreated soil	82.19	0.11	3.08	7.53	0.45	0.87	0.73	0.03

used a second time they were ignited and washed with 50 cc. of 0.05 *N* HCl, followed by 50 cc. of water. This method was found to give consistent results when solutions of known concentration were used. New filters were always washed with acid until the filtrate would give no blue color with Atkin's method, indicating phosphate or excessive silica. By following the procedure described in the foregoing and working with solutions of known phosphate content, it was established that no appreciable amounts of phosphorus were lost or gained as a result of absorption. Satisfactory results could be obtained regardless of the previous history of the filters.

DATA OBTAINED

In determining the soluble phosphate content of the soils, 25 gm. was weighed into a 500-cc. sterilizer bottle and 67.5 cc. of carbon dioxide free water was added. The bottles were shaken 4 hours on a horizontal shaker and then centrifugalized. Of the resultant suspension 50 cc. was filtered for the phos-

phorus determination, and the pH was determined on the remainder. Because of the small amount of suspension which was available for the pH determination, usually only about 1 cc. whereas the soil held about 16 cc., it was sometimes hard to obtain checks on duplicate samples and in certain cases the pH values are open to some question. In a few cases the pH data reported have been obtained in part by considering the results in other tables where

TABLE 2

Effect of $CaH_4(PO_4)_2$ on phosphorus solubility and pH

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

$CaH_4(PO_4)_2$, pounds per acre	Base soil was treated with															
	H		Fe		Al		None		Mg		Ca		NH_4		K	
	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH
0	0.20	2.97	0.19	3.64	0.15	3.88	0.21	4.77	0.19	5.19	0.20	5.17	0.62	5.97	0.59	6.00
100	0.24	2.97	0.20	3.64	0.16	3.85	0.22	4.77	0.20	5.22	0.24	5.24	1.65	6.02	2.58	5.80
250	0.40	2.97	0.20	3.64	0.17	3.85	0.26	4.77	0.41	5.21	0.77	5.30	3.65	6.07	6.35	6.00
500	0.72	2.97	0.20	3.64	0.16	3.61	0.78	4.82	1.56	5.05	1.64	5.34	4.82	6.00	8.17	5.94
750	1.36	2.97	0.20	3.64	0.17	3.58	2.21	4.62	2.02	5.15	1.62	5.39	7.02	6.07	9.31	5.84
1,000	2.36	2.97	0.20	3.64	0.17	3.56	4.13	4.75	2.19	5.12	2.21	5.40	9.35	6.02	10.32	5.77
1,250			0.23	3.49	0.46	3.99										
1,500			0.36	3.46	0.73	3.99										
2,000			0.48	3.36	0.86	4.04										

TABLE 3

Effect of $CaCO_3$ on phosphorus solubility and pH

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

$CaCO_3$, tons per acre	Base soil was treated with															
	H		Fe		Al		None		Mg		Ca		NH_4		K	
	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH
0	0.20	3.05	0.19	3.67	0.15	3.88	0.26	4.85	0.19	5.15	0.21	5.17	0.55	5.80	0.49	6.03
½	0.21	3.15	0.19	3.82	0.15	4.35	0.27	5.20	0.19	5.72	0.20	5.81	0.44	6.39	0.47	6.56
1	0.21	3.36	0.20	4.00	0.15	4.54	0.27	5.55	0.19	6.23	0.20	6.55	0.39	6.69	0.44	6.82
2	0.18	3.77	0.21	4.22	0.21	4.77	0.29	6.21	0.18	6.92	0.19	7.05	0.32	7.08	0.40	7.16
5	0.15	4.72	0.26	5.59	0.21	6.10	0.34	6.99	0.22	7.41	0.16	7.15	0.30	7.64	0.33	7.61
10	0.14	6.75	0.27	7.25	0.23	7.16	0.33	7.23	0.25	7.47	0.17	7.41	0.27	7.77	0.32	7.77

duplicate determinations were made. In the case of the NH_4 and K treated soils, with little or no fertilizer, it was hard to make the phosphorus determinations because of the masking of the blue color by dissolved organic matter. These determinations have been reported as found but have a larger probable error than the other determinations. By comparing the data in the first lines of tables 2, 3, 4, and 5 their reliability can be ascertained.

The effect of various fertilizers and soil amendments on phosphorus solubility, when the exchangeable bases varied in the soil, was determined and the data are reported in tables 2, 3, 4, and 5.

TABLE 4

Effect of $CaCl_2$ on phosphorus solubility and pH

Soil treated with various bases. Solubility expressed as parts per milion of filtrate. Soil water ratio 1 to 2½.

$CaCl_2$, Ca EQUIVALENT TO TONS $CaCO_3$ INDICATED PER ACRE	BASE SOIL WAS TREATED WITH															
	H		Fe		Al		None		Mg		Ca		NH_4		K	
	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH	P_2O_5	PH	P_2O_5	pH	P_2O_5	pH	P_2O_5	pH
0	0.18	3.19	0.18	3.56	0.15	3.63	0.21	4.70	0.18	5.05	0.20	5.00	0.55	5.81	0.46	5.75
½	0.18	3.19	0.18	3.27	0.15	3.56	0.19	4.65	0.17	4.98	0.16	5.00	0.40	5.75	0.45	5.65
1	0.17	3.15	0.17	3.27	0.14	3.56	0.19	4.59	0.15	4.98	0.16	5.13	0.22	5.06	0.40	5.58
2	0.17	3.01	0.19	3.25	0.14	3.55	0.20	4.59	0.15	4.98	0.15	5.17	0.16	4.54	0.36	5.78
5	0.16	2.92	0.17	3.20	0.14	3.55	0.20	4.54	0.14	4.82	0.15	4.97	0.15	4.40	0.28	5.32
10	0.15	2.82	0.18	2.99	0.14	3.37	0.19	4.50	0.13	4.88	0.15	4.84	0.14	4.22	0.24	5.19

TABLE 5

Effect of KCl on phosphorus solubility

Soil treated with various bases. Solubility expressed as parts per million of filtrate. Soil water ratio 1 to 2½.

KCl, POUNDS PER ACRE	BASE SOIL WAS TREATED WITH							
	H	Fe	Al	None	Mg	Ca	NH_4	K
0	0.20	0.21	0.14	0.27	0.19	0.19	0.55	0.49
100	0.18	0.19	0.14	0.24	0.19	0.18	0.55	0.46
250	0.18	0.17	0.14	0.24	0.16	0.19	0.55	0.41
500	0.17	0.18	0.14	0.23	0.20	0.18	0.55	0.40
750	0.20	0.16	0.13	0.21	0.19	0.17	0.56	0.43
1,000	0.20	0.21	0.14	0.23	0.20	0.18	0.56	0.37

DISCUSSION

An examination of these data shows conclusively that the cation absorbed by the soil complex greatly affects the solubility of phosphorus. The first lines in tables 2, 3, 4, and 5 show that in using the untreated soil as a standard, the replacement of the absorbed bases by Al, Fe, H, Mg, and Ca decreases the soluble phosphorus, and that replacement of the bases with NH_4 or K increases the soluble phosphorus. Apparently, under the conditions governing our work, absorbed aluminum precipitates most completely the soil phosphorus, followed by magnesium and iron and then hydrogen and calcium. It is probable that the precipitation caused by hydrogen is due to iron or aluminum which has been dissolved by the high degree of acidity. It may be noted that

with various bases held by the absorbing complex of the soil, the soluble phosphorus remains quite constant and the pH varies considerably. In particular note the H, Fe, Mg, and Ca soils which, unfertilized, have approximately 0.20 p.p.m. of soluble phosphorus, and whose pH values vary from about 3.00 to about 5.00. The results obtained as to the effect of absorbed base agree with the findings of Comber (4), Smirnov (11), Spurway (12), and Kappen and Bollenbeck (7).

The data in table 2 show that for soils treated with Al and Fe it is necessary to add 1,250 and 1,500 pounds of $CaH_4(PO_4)_2$ per acre before the soluble P_2O_5 in the soil is appreciably increased. That this is attributable to absorbed base rather than reaction is indicated by the H soil, which, although somewhat more acid in reaction than the Al or Fe soils, yet shows an appreciable increase in soluble phosphate with an application of 250 pounds of $CaH_4(PO_4)_2$ per acre. It should be noted in table 2 that applications of 1,250 pounds per acre of $CaH_4(PO_4)_2$ to the Al soils or even 2,000 pounds to the Fe soil did not result in as much soluble phosphate as was present in the unfertilized NH_4 and K soils, even though these fertilizer treatments increased the phosphate content of the soils by 34 per cent and 55 per cent respectively. That the hydrogen, calcium, and magnesium soils also precipitate the phosphorus is evident from tables 2, 3, 4, and 5. In the case of these three soils, the data in table 2 show that 250 pounds per acre of $CaH_4(PO_4)_2$ is required to increase the soluble phosphorus appreciably, and around 250 to 500 pounds per acre is required to bring it up to that of the unfertilized NH_4 and K soils. An application of 100 pounds per acre, the smallest application made, greatly increased the soluble phosphate content of the NH_4 and K soils.

It will be seen from the data that, excepting the hydrogen and untreated soils, the more alkaline the absorbed base makes the soil, the more soluble is the phosphorus in that soil, and the less precipitating power does that soil have for added phosphorus. The power of the soils containing various exchangeable bases to precipitate P_2O_5 agrees with the work of Nemec (8) and Nemec and Gracinin (9).

In table 3 are presented the data resulting from the studies of the effect of applications of calcium carbonate to the soils. In the cases of the H, Ca, NH_4, and K soils the $CaCO_3$ decreases the solubility of the phosphorus. Theoretically this would be caused by the substituting of a more insoluble calcium phosphate for a more soluble calcium phosphate, or the H, NH_4, or K phosphates. The substitution of a more insoluble calcium phosphate might be replacing a dibasic salt by a tribasic or a dibasic salt for a monobasic. In the case of the Fe, Al, Mg, and untreated soils the applications of $CaCO_3$ have increased the solubility of the soil phosphorus. This increased solubility might be caused by the precipitating of the Al and Fe ions by the basic reaction, and the substituting of the more soluble calcium phosphates for the less soluble phosphates of iron or aluminum. In every case the addition of calcium carbonate has made the soil more basic. This effect of $CaCO_3$ increasing the

solubility of phosphorus agrees quite well with the work Askinazi and Yarusov (2), Wityn (15), Antanov (1), and Demolon and Barbier (5).

That the increased solubility of P_2O_5 in the case of Fe, Al, Mg, and untreated soils is due to the change in reaction rather than to the presence of the calcium, is made evident by comparing the data in tables 3 and 4. In table 4 the results of the application of calcium chloride instead of calcium carbonate are presented. By this treatment the calcium was applied in a vastly more soluble form, as a salt that would have a very small effect on the soil reaction. The applications of $CaCl_2$ contained calcium equivalent to the calcium added in the carbonate form. In no case where the calcium chloride was added did an increase in phosphorus solubility result. With the H, Fe, Al, untreated, Mg, and Ca soils $CaCl_2$ had little or no effect on phosphorus solubility, while in the case of the NH_4 and K soils $CaCl_2$ materially decreased the solubility. In all cases application of $CaCl_2$ rendered the reaction somewhat more acid. From the data in tables 3 and 4 it appears that applications of calcium are either inactive or tend to precipitate the phosphorus in a soil. If, however, the calcium is applied in a form that increases the alkalinity, the phosphorus solubility might be increased, probably as a result of secondary reactions.

In view of the fact that theoretically potassium fertilization might relieve phosphorus deficiencies by increasing the solubility of the soil phosphorus due to a non-common ion, the effect of applications of potassium chloride has been investigated with the eight soils. The results reported in table 5 show little or no effect on phosphorus solubility with applications of KCl up to 1,000 pounds per acre. The physico-chemical law that the addition of an unlike ion will increase the solubility of the ions already present would lead one to expect an increased phosphorus solubility. This result was not found when working with Cherokee loam, regardless of the base contained in the soil.

Attention should be called to the data in table 3. Here it is seen that with heavy applications of lime, more soluble P_2O_5 appears in the iron and aluminum soils than in the hydrogen soil. These data have been re-checked and a further investigation of these solubility effects is in progress.

SUMMARY AND CONCLUSIONS

The absorbed bases in separate portions of Cherokee loam were replaced with H, Fe, Al, Ca, Mg, K, and NH_4, and an untreated portion was also retained. The eight soils thus prepared were treated with $CaH_4(PO_4)_2$, $CaCO_3$, $CaCl_2$, and KCl in varying amounts, and the water-soluble phosphorus was determined.

The soluble phosphorus of soil is increased by replacing the absorbed bases by K or NH_4.

When phosphorus is applied as $CaH_4(PO_4)_2$ the Fe and Al soils precipitate large amounts of phosphorus; the H, Mg, Ca soils, intermediate amounts; and the K and NH_4 soils, small amounts.

In acid soils with similar pH values, less phosphorus will be precipitated on the application of phosphatic fertilizers when the acidity is caused by absorbed hydrogen rather than by the hydrolysis.

$CaCO_3$ applications increase the soluble phosphorus in the Fe, Al, and Mg soils, but decrease it in the H, Ca, NH_4, and K soils.

Phosphorus solubility is increased by lime on soils made acid by absorbed Fe or Al, but is decreased on soils made acid by absorbed H.

The increased phosphorus solubility resulting from lime is attributed to the reaction rather than to the calcium, since applications of $CaCl_2$ failed to increase the solubility.

The solubility of the phosphorus in a soil is not affected by the application of KCl, regardless of the base held by the soil.

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A SIMPLE AND RAPID METHOD FOR MEASURING THE STICKINESS OF SOILS[1]

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In previous communications (2, 4) studies have been reported on the relationship that exists between certain physical properties of soils, such as moisture equivalent, unfree water, heat of wetting, flowing point, upper plastic limit, and crumbling point, and their clay and colloidal content as determined by the hydrometer method (3) of mechanical analysis.

Stickiness is the next physical property of soils whose relationship to clay and colloidal content it was undertaken to investigate. This property is prominent in nearly all of the fine textured soils and has a great bearing on their tilth, resistance to crushing, water percolation, and handling. It was found, however, that there was no satisfactory method for measuring quantitatively the degree of stickiness in soils and hence it was necessary to devise a method. After some experimentation a simple and rapid method was devised which gave a satisfactory quantitative measurement of the maximum stickiness in different soils. It is the object of this paper to present this new method and the experimental results obtained with it.

METHOD AND PROCEDURE

The principle of the method consists in pressing a small metal disk against the surface of a thoroughly puddled and kneaded soil, and then pulling this metal disk vertically upward by means of a spring balance upon which the force of pull is directly recorded. The moisture content of the soil is gradually varied until the maximum pull is obtained.

The following pieces of apparatus are required for making the determination: a metal disk, a recording spring balance, two spatulas, a base, a pan, a pipette, and a balance for weighing soil.

The metal disk is made of sheet copper 2 inches in diameter and $\frac{1}{16}$ inch in thickness and has a small ring exactly in the center of the upper surface. The total area of the disk is 3.14 square inches. The metal is of sufficient thickness and strength not to bend.

The spring balance is vertical and reads to 30 pounds in $\frac{1}{4}$-pound divisions and 15 kilos in 100-gm. divisions. It has a sliding recorder which remains at

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the maximum pull reached after the spring is released and the indicator goes back to zero. This spring balance together with the metal disk is shown in plate 1.

Two spatulas are required, one with a thin blade that will bend easily and one with a stiff blade that will not bend. The flexible spatula is used mainly in gathering the soil into a mass or mound, and the non-flexible blade is employed principally in puddling and working the soils to reduce them to the particle state, and to develop their stickiness.

For a base it was found most desirable and convenient to use an iron block 5 inches square, 4 inches deep, with a rather smooth surface and weighing about 35 pounds. This kind of base makes it much easier to work the soils and to press the metal disk down on them.

The pan is used to mix the dry soils with water at the beginning so that none will be lost. After the soils are moistened they are transferred to the iron block where all subsequent mixing and working are done.

The procedure adopted consists in weighing out 30 gm. of air-dry soil which has passed through a 2-mm. sieve, spreading it in a thin layer in the pan, and adding a sufficient quantity of distilled water from a graduated pipette to make it just moist enough so it can be kneaded. After being thoroughly mixed the soil is removed to the iron block and worked vigorously with the spatula to obtain as complete dispersion as possible. Various movements can be employed to knead and disperse the soil, but at the lowest moisture content the one that seems to be the easiest and most efficient is to slice the soil into thin layers by a shearing stroke of the spatula and press it or rub it on the iron surface. The soil is again gathered into a mass or mound and the process of kneading and working is repeated many times until the soil is thoroughly dispersed. Finally, the whole soil mass is gathered into a mound by the spatulas and the metal disk is pressed on it with considerable force until there is an intimate and complete contact between the surface of the disk and the soil. The spring balance is then hooked through the ring on the metal disk and a gradual, vertical pull exerted, the balance being held firmly between the hands, until the disk breaks away from the soil. The pul required to break the contact is recorded. The soil is gathered into a mound again and the determination repeated several times in order to obtain a series of readings from which to get an average. If the testing is carefully done the determinations will agree closely. In case they do not agree the soil is insufficiently dispersed or sufficient care has not been exercised to obtain complete contact between the disk and soil. To the same soil sample is then added 1 cc. more water and the process of mixing, kneading, gathering it into a mound, and determining the pull required to break the disk away from the soil is repeated. The whole process is repeated until a maximum pull is reached and passed. It usually takes about an hour to determine the maximum stickiness of most soils.

For a satisfactory application of this method the following essential points

must be strictly observed: (*a*) The soil should be as completely dispersed as possible in order to develop its potential maximum stickiness. This end is undoubtedly accomplished by using the same soil sample in all the different determinations, and by starting with a low moisture content at which a soil can be more effectively dispersed than at a high moisture content where the compound particles slide easily over one another. (*b*) The metal dish should be pressed with sufficient force, which is very considerable at the lower soil moisture contents, to produce a very intimate contact between its surface and the soil. On the other hand, extreme care must be taken not to press the disk down so hard that it displaces most of the soil, and allows its surface to come very nearly in contact with the surface of the base. When this is done another external factor comes into play which tends to vitiate the results. It is only at the higher moisture contents when the soil is very soft, however, that it is easily displaced. (*c*) The surface of the disk should be cleaned with a wet cloth and wiped dry every time the disk is used, especially at the lower moisture contents. If soil dries on the disk, especially in the form of dust, it seems to prevent the moist soil sticking to the disk. In this connection it might be said that fingers are not especially good objects with which to test stickiness. (*d*) The disk should be pressed down as level as possible and should be pulled up straight and gradually, and not with a jerk. (*e*) It is advisable to gather the soil into a high mound and the press then disk down on it until the surface of the soil equals that of the disk.

When the disk is pulled up, the break almost always takes place in the soil mass and not between the surface of the disk and the soil. Soils at very low moisture content, and soils possessing little stickiness may be exceptions to this rule.

Since the same soil sample is employed for all determinations, the moisture content at each measurement can only be approximately calculated from the amount of water added. This, however, is of no serious importance since a knowledge of the exact water content is not essential.

Although the method cannot be considered to be highly accurate and sometimes tends to give somewhat erratic results, yet by following the technique as developed and by observing the precautions mentioned, fairly satisfactory results are obtained.

The term "stickiness" is employed here to include all the forces that the conventional terms "adhesion" and "cohesion" are supposed to represent. Critical examination tends to show that there is no fundamental difference (1) between adhesion and cohesion and especially in soils which are not a homogeneous mass but rather a heterogeneous mass with water playing a leading rôle in the development and manifestation of these forces.

EXPERIMENTAL RESULTS

In table 1 are presented the experimental results on the stickiness of a large number of representative types of soil. The data are expressed in pounds

TABLE 1

Stickiness of Different Soils in Pounds Pull Per Square Inch at Various Water Contents; with Special Emphasis on the Maximum Stickiness

SOILS	STICKINESS IN POUNDS PULL PER SQUARE INCH AT MOISTURE PERCENTAGES OF																		
	20.0 %	23.3 %	26.6 %	30.0 %	33.3 %	37.0 %	40.0 %	43.3 %	46.6 %	50.0 %	53.3 %	56.6 %	60.0 %	63.3 %	66.6 %	70.0 %	73.3 %	76.6 %	80.0 %	83.5 %
1. Muck	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2. Plainfield sand, surface	0.08	0.09	0.07																	
3. Dickson sandy loam surface	1.0	1.2	1.0	0.80																
4. Clarion loam, subsoil	0.692	0.89	0.40																	
5. Clarion loam, surface			2.39	3.18	3.34	2.86	2.07													
6. Lindy silt loam, surface		1.41	2.89	2.98	2.82	2.20	1.70													
7. Clinton silt loam, surface				1.80	2.67	2.20	1.25													
8. Putman silt loam, surface						2.70	3.10	2.80	2.00	1.30										
9. Grundy silt loam, surface			1.91	2.55	3.19	2.86	2.55	1.59												
10. Edena silt loam, 0–9 inches					2.67	3.30	3.92	3.77	3.19	2.83										
11. Antioch clay loam, 40–60 inches	3.49	4.08	4.73	5.34	5.18	4.24	3.30	2.51												
12. Almont adobe, 0–10 inches			4.30	5.10	5.41	5.57	5.73	4.46	3.02	3.82	3.82	3.19	2.71	2.55	2.39	2.07				
13. Susquehanna clay, 16–20 inches			2.55	3.50	4.30	4.01	4.01	4.01	3.82	3.82										
14. Davidson loam, 18–30 inches											2.83	2.98	2.83	2.83	2.83	2.51	2.51			
15. Decatur clay, 6–12 inches				4.46	4.62	4.81	4.30	3.82	3.19											
16. Ontonagon clay, C horizon				5.18	6.28	6.91	6.44	5.50	4.55											
17. Haldemand clay, subsoil					4.50	5.49	5.81	5.65	4.08	3.49										
18. Capay clay, adobe		5.57	6.85	7.49	7.96	7.80	7.80	6.85	6.68	6.37	5.73	5.10	3.53							
19. Lake Charles clay, surface			3.96	5.10	5.73	5.48	3.02													
20. Fargo silty clay, subsoil						5.78	6.30	6.90	7.50	7.45	7.40	7.20	7.00	6.40	6.0	5.1				
21. Fargo clay, 6–16 inches				3.19	3.49	5.34	7.54	7.54	7.69	7.85	7.69	7.54	6.12	5.02	4.40					
22. Fulton clay, subsoil				5.43	6.30	7.0	7.10	7.0	6.5	6.0										
23. Fargo silty clay, 3–8 inches							7.07	7.20	7.50	7.80	7.50	7.01	6.80	5.30	4.50					
24. Black clay, adobe					5.35	6.50	6.80	7.20	6.90	6.80	5.60	5.00	4.33							
25. McKenzie clay, B_3					5.88	7.90	7.98	8.12	8.45	8.92	8.99	9.30	9.30	9.05	9.01	8.70	8.0	7.60	6.30	5.50
26. Soils 8 and 25 mixed half and half				5.75	6.80	7.50	7.70	7.90	7.80	7.30	6.50	5.80	4.90							
27. Mixture $\frac{1}{3}$ soil 25 and $\frac{2}{3}$ soil 8					4.40	6.0	6.20	5.80	5.05	4.20	3.60									
28. Soils 8 and 16 mixed half and half				3.98	5.20	4.90	3.82	2.90	2.58											
29. Soils 2 and 25 mixed half and half			8.84	9.00	9.12	8.50	8.10	7.17	6.50	5.57	4.50									
30. Mixture $\frac{2}{3}$ soil 2 and $\frac{1}{3}$ soil 25	6.98	7.94	7.27	7.01	6.05	4.46	3.18													
31. Soils 1 and 25 mixed half and half									2.90	4.80	5.10	5.50	5.55	5.10	4.90	4.01	3.60			
32. Mixture $\frac{1}{3}$ soil 1 and $\frac{2}{3}$ soil 25								5.09	6.70	6.98	7.40	7.80	7.40	6.80	5.90	5.01	4.60			

pull per square each. Each measurement is the average of five or more trials. The data in table 1 show that the property of stickiness varies greatly both in different soils and within the same soil at different moisture contents. In the muck, sands, and sandy loams tested there is no measurable degree of stickiness at any moisture content, but in the loams and especially in the clays there is comparatively a very high degree of stickiness over a wide range of moisture content. Stickiness tends to increase more or less with the moisture content until a maximum is reached and then it decreases. Every soil appears to have a definite maximum degree of stickiness, the magnitude of which ranges from 0.0 pounds pull per square inch in muck to 0.09 pounds in Plainfield sand; 3.10 pounds in Putman silt loam; 5.73 pounds in Lake Charles clay; 7.50 pounds in Fargo silty clay; and 9.30 pounds in McKenzie clay. The last figure, which is the largest obtained in the soils investigated, represents really a tremendous amount of stickiness.

It will be observed that the maximum stickiness remains practically the same for a considerable range of moisture in nearly all of the heavy soils but especially McKenzie clay, Davidson loam, and Fargo clay loam. This is probably due to the fact that as the soils are being dispersed their consistency tends to remain almost the same for a considerable range of moisture content until they are completely dispersed. This explanation finds support in the previous work on the flowing point of soils (4). When the soils are not completely dispersed an apparent maximum stickiness is reached more abruptly but the result may not be the maximum stickiness which it is possible to develop in the soil.

The results for soils 14, 29, 30, 31 and 32 deserve special attention. Soil 14, which is Davidson loam and a laterite, contains 71.2 per cent clay and yet has very little stickiness. The same is true of Nipe clay, which is a more typical laterite than the Davidson loam. Apparently lateritic soils possess very little stickiness. Soil 29 is a mixture of one part of Plainfield sand and one part of McKenzie clay, and soil 30 contains two parts of Plainfield sand and one part of McKenzie clay, yet these mixtures manifest as high stickiness as the McKenzie clay alone. In other words, diluting this extremely sticky soil with two parts of sand to one part of clay does not perceptibly decrease its maximum degree of stickiness. On the other hand, diluting this same McKenzie clay with muck causes its stickiness to be markedly reduced as shown by the result for soils 31 and 32, which contain one part of muck to one part of clay, and one part of muck to two parts of clay, respectively. Apparently muck has a great influence in reducing stickiness whereas sand has practically none, at least, in the proportions tried.

RELATIONSHIP BETWEEN MAXIMUM STICKINESS AND THE CLAY CONTENT OF SOILS

After the maximum degree of stickiness was determined in the different soils, it was decided to ascertain what relationship this property bears to the clay (0.005 — 0 mm.) and clay (0,002 — 0 mm.) as determined by the hydrometer method (3). This relationship is revealed in table 2.

From the last two columns of table 2, which show the ratio between maximum stickiness and clay and fine clay, it is readily seen that out of 22 soils examined, 20 show fairly close relationship between their maximum degree of stickiness and their clay and fine clay content. The two exceptions are Davidson loam, which is a laterite, and Haldemand clay subsoil.

The results of table 2 indicate that, on the whole, stickiness is controlled mainly by the clay and colloidal content, although in certain cases some other factor, possibly composition, is also a determining factor. In lateritic soils there is distinctly no relationship between their stickiness and clay content.

TABLE 2

Comparison Between Maximum Stickiness and Clay Content of Soils

SOILS	MAXIMUM STICKINESS	CLAY	FINE CLAY	RATIO CLAY / STICKINESS	RATIO FINE CLAY / STICKINESS
	pounds	*per cent*	*per cent*	*ratio*	
Dickinson sandy loam, surface	1.2	13.5	12.5	11.3	10.4
Clarion loam, surface	3.34	24.3	21.8	7.28	6.53
Lindy silt loam, surface	2.89	25.7	20.6	8.90	7.13
Clinton silt loam, surface	2.67	29.2	23.4	10.9	8.76
Putman silt loam, surface	3.10	24.2	20.5	7.81	6.61
Grundy silt loam, surface	3.19	33.2	29.2	10.4	9.16
Edena silt loam, 0–9 inches	3.92	36.1	30.7	9.21	7.83
Antioch clay loam, 40–60 inches	5.34	34.8	32.4	6.52	6.06
Almont adobe, 0–10 inches	5.73	51.8	45.9	9.04	8.01
Susquehanna clay, 16–20 inches	4.30	41.0	39.5	9.54	9.18
Davidson loam, 18–30 inches	2.98	71.2	69.1	23.9	23.2
Decatur clay, 6–12 inches	4.81	42.3	40.7	8.80	8.46
Ontonagon clay, C	6.91	79.8	72.7	11.6	10.50
Haldemand clay, subsoil	5.81	85.6	77.4	14.7	13.3
Capay clay, adobe	7.96	63.2	56.9	7.94	7.15
Lake Charles clay, surface	5.73	42.4	38.9	7.40	6.79
Fargo silty clay, subsoil	7.50	69.7	64.6	9.29	8.63
Fargo clay, 6–16 inches	7.85	60.4	55.1	7.69	7.02
Fargo silty clay, 3–8 inches	7.80	70.2	65.8	9.00	8.45
Fulton clay, subsoil	7.10	68.0	66.5	9.58	9.36
Black clay, adobe	7.20	55.9	52.7	7.76	7.32
McKenzie clay, B_2	9.30	76.1	71.9	8.18	7.73

High organic matter content especially in undecomposed forms reduces markedly the stickiness. Carbonates do not seem to exert much effect, judging from the results from Ontonagon clay, C horizon, and Fulton clay subsoil, both of which contain a very high content of carbonates but in the colloidal size.

RELATIONSHIP BETWEEN MAXIMUM STICKINESS AND UPPER PLASTIC LIMIT

In order to determine what relationship exists between the upper plastic limit of soils and their maximum stickiness, a comparison was made of the two properties and is presented in table 3.

From the results in table 3 and especially those in the last column, it is apparent that for the majority of the soils investigated there is some, though not a very close, relationship between their maximum stickiness and upper plastic limit. In the case of certain soils, particularly the lateritic Davidson loam, no relation appears to exist between these two properties.

TABLE 3

Comparison Between Maximum Stickiness and Upper Plastic Limit of Soils

SOILS	UPPER PLASTIC LIMIT	MAXIMUM STICKINESS	RATIO $\frac{\text{U.P.L.}}{\text{M.S.}}$
	per cent	*pounds*	
Lindy silt loam, surface	27.2	2.89	9.41
Clinton silt loam, surface	29.9	2.67	11.30
Putman silt loam, surface	37.4	3.10	12.00
Clarion loam, surface	31.5	3.34	9.43
Edena silt loam, 0–9 inches	39.5	5.34	7.40
Almont adobe, 0–10 inches	39.1	5.73	6.82
Susquehanna clay, 16–20 inches	46.0	4.30	10.70
Decatur clay, 6–12 inches	32.0	4.81	6.65
Ontonagon clay, C	36.3	6.91	5.25
Lake Charles clay, surface	39.6	5.73	6.91
Davidson loam, 18–30 inches	59.8	2.98	20.10
Haldemand clay, subsoil	41.3	5.81	7.11
Capay clay, adobe	41.80	7.96	5.25
Fulton clay, subsoil	39.8	7.10	5.60
McKenzie clay, B_2	71.1	9.30	7.64

SUMMARY

A simple, rapid, and fairly accurate method has been developed to measure quantitatively the maximum stickiness in soils.

The results obtained show that this maximum degree of stickiness measured in pounds per square inch required to pull a metal disk from the soil, varies from 0.0 pounds in muck and sand to about 10 pounds in some of the clays.

There is a fairly close relationship between the maximum stickiness and clay content in most soils, but in some soils this relationship is very slight.

There is also some, though not a close, relationship between the maximum stickiness and the upper plastic limit in most soils, but there are some very distinct exceptions.

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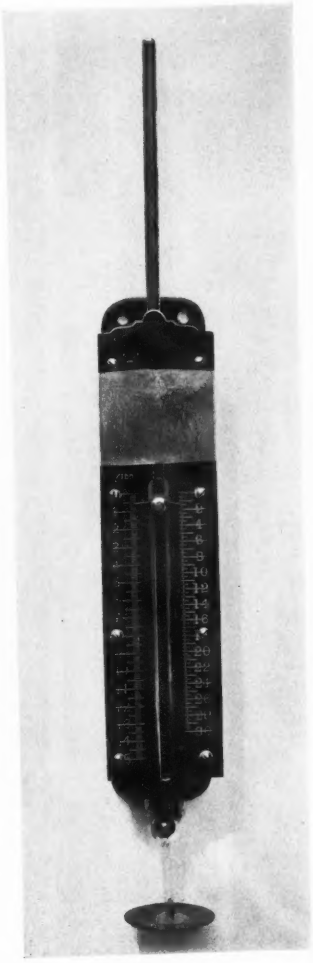
PLATE 1

SPRING BALANCE AND DISK FOR MEASURING THE STICKINESS OF SOILS



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